8114056

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

May 24, 2021

THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF THE FILE WRAPPER AND CONTENTS OF:

APPLICATION NUMBER: *16/397,267*
FILING DATE: *April 29, 2019*
PATENT NUMBER: *10888601*
ISSUE DATE: *January 12, 2021*

Certified by

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

Electronically Filed

PRELIMINARY AMENDMENT Under CFR 1.115 Address to: Mail Stop Patent Application Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450		
	Attorney Docket No.	REGN-008CIPCON5
	Confirmation No.	To Be Assigned
	First Named Inventor	YANCOPOULOS, GEORGE D.
	Application Number	To Be Assigned
	Filing Date	April 29, 2019
	Group Art Unit	To Be Assigned
	Examiner Name	To Be Assigned
	Title: *"Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders"*	

Sir:

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

Electronically Filed

NOTIFICATION OF PRIOR SEQUENCE LISTING		
	Attorney Docket	REGN-008CIPCON4
	First Named Inventor	YANCOPOULOS, GEORGE D.
	Application Number	To Be Assigned
	Filing Date	April 29, 2019
Address to: Mail Stop Patent Application Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Confirmation Number	To Be Assigned
	Group Art Unit	To Be Assigned
	Examiner Name	To Be Assigned
	Title: "USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS"	

Sir:

The above-identified patent application contains sequences of nucleic acid and polypeptides. A sequence listing was prepared for parent application, **16/159,282,** filed **October 12, 2018**, in paper and computer-readable format. The sequence information in the paper or compact disk copy of the sequence listing (required by 1.821(c)) of this application is identical to the sequence information in the computer-readable format (CRF) of the above-identified other application. No new matter has been added. Therefore, please transfer to this application, in accordance with 37 CFR § 1.821(e), the fully compliant computer readable copy from applicants' other application. A paper (.txt) copy of this sequence listing is enclosed.

Applicants respectfully submit that the present patent application is now in compliance with 37 CFR §§ 1.821 - 1.825. The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number REGN-008CIPCON5.

Respectfully submitted,

BOZICEVIC, FIELD & FRANCIS LLP

Dated: 29 April 2019

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

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

SEQUENCE LISTING

<110> George D. Yancopoulos

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

<130> 725A1

<140> To be assigned
<141> Filed herewith

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

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Mylan Exhibit 1017
Mylan v. Regeneron, IPR2022-01226
Page 6

Samsung Bioepis Exhibit 1017, pg. 6

Electronic Patent Application Fee Transmittal				
Application Number:				
Filing Date:				
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS			
First Named Inventor/Applicant Name:	George D. YANCOPOULOS			
Filer:	Karl Bozicevic/Kimberly Zuehlke			
Attorney Docket Number:	REGN-008CIPCON5			
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:				
UTILITY APPLICATION FILING	1011	1	300	300
UTILITY SEARCH FEE	1111	1	660	660
UTILITY EXAMINATION FEE	1311	1	760	760
Pages:				
Claims:				
CLAIMS IN EXCESS OF 20	1202	9	100	900
INDEPENDENT CLAIMS IN EXCESS OF 3	1201	1	460	460
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**3080**

Electronic Acknowledgement Receipt	
EFS ID:	35858839
Application Number:	16397267
International Application Number:	
Confirmation Number:	8135
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-008CIPCON5
Receipt Date:	29-APR-2019
Filing Date:	
Time Stamp:	14:51:44
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$3080
RAM confirmation Number	043019INTEFSW14522800
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	REGN-008CIPCON5_2019-04-29_ADS.pdf	1256704 0e88f782022f8326bee7e55bd6957f75e862209c	no	9
Warnings:					
Information:					
2		REGN-008CIPCON5_2019-04-29_Appln_as_fld.pdf	155991 85273a495296621a979ffed2ccdec6502c799c0c	yes	24

Multipart Description/PDF files in .zip description

Document Description	Start	End
Abstract	24	24
Claims	22	23
Specification	1	21

Warnings:					
Information:					
3	Drawings-only black and white line drawings	REGN-008CIPCON5_Figure.pdf	105393 2d582f645d0c5d17d717e589b029a39331991bdb	no	1
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The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
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The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
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5		REGN-008CIPCON5_2019-04-29_pre_amend_asfld.pdf	46449 ddb00b729b9b88f23f25609bea2b88928efdfd95	yes	7
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Applicant Arguments/Remarks Made in an Amendment		6	7	
	Claims		2	5	
	Preliminary Amendment		1	1	
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Information:					
6	Sequence Listing	REGN-008CIPCON5_2019-04-29_seq_list_trans.pdf	27214 a9c529b4a455314e4c569d3545204027bacf5d68	no	1
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Information:					
		Total Files Size (in bytes):	1828026		

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

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON5
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

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

Inventor Information:

Inventor 1 — Remove

Legal Name

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

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

City	State/Province	Country of Residence
Yorktown Heights	NY	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.
Address 2	777 Old Saw Mill River Road

City	State/Province	Postal Code	Country
Tarrytown	NY	10591	US

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

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	96387		
Email Address	docket@bozpat.com	Add Email	Remove Email

Application Information:

Title of the Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS		
Attorney Docket Number	REGN-008CIPCON5	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	1	**Suggested Figure for Publication (if any)**	1

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON5
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Filing By Reference:

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

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

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

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

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

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	● Customer Number	US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	96387		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

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

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON5
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Prior Application Status	Patented			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
16159282	Continuation of	15471506	2017-03-28	10130691	2018-11-20
Prior Application Status	Patented			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15471506	Continuation of	14972560	2015-12-17	9669069	2017-06-06
Prior Application Status	Patented			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
14972560	Continuation of	13940370	2013-07-12	9254338	2016-02-09
Prior Application Status	Expired			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)	
13940370	Continuation in part of	PCT/US2012/020855	2012-01-11	
Prior Application Status	Expired			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)	
PCT/US2012/020855	Claims benefit of provisional	61432245	2011-01-13	
Prior Application Status	Expired			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)	
PCT/US2012/020855	Claims benefit of provisional	61434836	2011-01-21	
Prior Application Status	Expired			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)	
PCT/US2012/020855	Claims benefit of provisional	61561957	2011-11-21	
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.				Add

Foreign Priority Information:

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON5
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

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

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

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

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

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON5
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Authorization or Opt-Out of Authorization to Permit Access:

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

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

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

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

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

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

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

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

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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON5
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

Applicant Information:

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

Applicant 1 Remove

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

Clear

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

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

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	REGENERON PHARMACEUTICALS, INC.		
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Phone Number		Fax Number	
Email Address			

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

Assignee Information including Non-Applicant Assignee Information:

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Assignee 1

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Organization Name	REGENERON PHARMACEUTICALS, INC.

Mailing Address Information For Assignee including Non-Applicant Assignee:

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

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

Signature	/Karl Bozicevic, Reg. No. 28,807/			Date (YYYY-MM-DD)	2019-04-29
First Name	Karl	Last Name	Bozicevic	Registration Number	28807

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-008CIPCON5
	Application Number	
Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS	

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

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

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ABSTRACT

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

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What is claimed is:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of 16/159,282 filed October 12, 2018, which is a continuation of 15/471,506 filed March 28, 2017, now U.S. Patent No. 10,130,691 issued November 20, 2018, which is a continuation of 14/972,560 filed December 17, 2015, now U.S. Patent No. 9,669,069 issued June 6, 2017, which is a continuation of 13/940,370 filed July 12, 2013, now U.S. Patent No. 9,254,338 issued February 9, 2016, which is a continuation-in-part of International Patent Application No. PCT/US2012/020855, filed on January 11, 2012, which claims the benefit of US Provisional Application Nos. 61/432,245, filed on January 13, 2011, 61/434,836, filed on January 21, 2011, and 61/561,957, filed on November 21, 2011, the contents of which are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

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

BACKGROUND

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

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

[0005] Methods for treating eye disorders using VEGF antagonists are mentioned in, *e.g.*, US 7,303,746; US 7,306,799; US 7,300,563; US 7,303,748; and US 2007/0190058. Nonetheless, there remains a need in the art for new administration regimens for angiogenic eye disorders, especially those which allow for less frequent dosing while maintaining a high level of efficacy.

BRIEF SUMMARY OF THE INVENTION

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

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

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

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

[0010] Aflibercept (EYLEA™, Regeneron Pharmaceuticals, Inc) was approved by the FDA in November 2011, for the treatment of patients with neovascular (wet) age-related macular degeneration, with a recommended dose of 2 mg administered by intravitreal injection every 4 weeks for the first three months, followed by 2 mg administered by intravitreal injection once every 8 weeks.

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

BRIEF DESCRIPTION OF THE FIGURE

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

DETAILED DESCRIPTION

[0013] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0014] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0015] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

DOSING REGIMENS

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

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

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

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

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

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embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0021] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 4 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 8 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. For example, the present invention includes methods which comprise administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by at least 5 tertiary doses of the VEGF antagonist, wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered from 8 to 12 (*e.g.*, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12) weeks after the immediately preceding dose. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

VEGF ANTAGONISTS

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

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

[0024] VEGF receptor-based chimeric molecules include chimeric polypeptides which comprise two or more immunoglobulin (Ig)-like domains of a VEGF receptor such as VEGFR1 (also referred to as Flt1) and/or VEGFR2 (also referred to as Flk1 or KDR), and may also contain a multimerizing domain (*e.g.*, an Fc domain which facilitates the multimerization [*e.g.*, dimerization] of two or more chimeric polypeptides). An exemplary VEGF receptor-based chimeric molecule is a molecule referred to as VEGFR1R2-FcΔC1(a) which is encoded by the nucleic acid sequence of SEQ ID NO:1. VEGFR1R2-FcΔC1(a) comprises three components: (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130 to

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231 of SEQ ID NO:2; and (3) a multimerization component ("FcΔC1(a)") comprising amino acids 232 to 457 of SEQ ID NO:2 (the C-terminal amino acid of SEQ ID NO:2 [*i.e.*, K458] may or may not be included in the VEGF antagonist used in the methods of the invention; *see e.g.*, US Patent 7,396,664). Amino acids 1-26 of SEQ ID NO:2 are the signal sequence.

[0025] The VEGF antagonist used in the Examples set forth herein below is a dimeric molecule comprising two VEGFR1R2-FcΔC1(a) molecules and is referred to herein as "VEGFT." Additional VEGF receptor-based chimeric molecules which can be used in the context of the present invention are disclosed in US 7,396,664, 7,303,746 and WO 00/75319.

ANGIOGENIC EYE DISORDERS

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

PHARMACEUTICAL FORMULATIONS

[0027] The present invention includes methods in which the VEGF antagonist that is administered to the patient is contained within a pharmaceutical formulation. The pharmaceutical formulation may comprise the VEGF antagonist along with at least one inactive ingredient such as, *e.g.*, a pharmaceutically acceptable carrier. Other agents may be incorporated into the pharmaceutical composition to provide improved transfer, delivery, tolerance, and the like. The term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the antibody is administered. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, Pa., 1975), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as

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LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in the context of the methods of the present invention, provided that the VEGF antagonist is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Powell et al. PDA (1998) J Pharm Sci Technol. 52:238-311 and the citations therein for additional information related to excipients and carriers well known to pharmaceutical chemists.

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

MODES OF ADMINISTRATION

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

AMOUNT OF VEGF ANTAGONIST ADMINISTERED

[0030] Each dose of VEGF antagonist administered to the patient over the course of the treatment regimen may contain the same, or substantially the same, amount of VEGF antagonist. Alternatively, the quantity of VEGF antagonist contained within the individual doses may vary over the course of the treatment regimen. For example, in certain embodiments, a first quantity of VEGF antagonist is administered in the initial dose, a second quantity of VEGF antagonist is administered

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in the secondary doses, and a third quantity of VEGF antagonist is administered in the tertiary doses. The present invention contemplates dosing schemes in which the quantity of VEGF antagonist contained within the individual doses increases over time (*e.g.*, each subsequent dose contains more VEGF antagonist than the last), decreases over time (*e.g.*, each subsequent dose contains less VEGF antagonist than the last), initially increases then decreases, initially decreases then increases, or remains the same throughout the course of the administration regimen.

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

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

TREATMENT POPULATION AND EFFICACY

[0033] The methods of the present invention are useful for treating angiogenic eye disorders in patients that have been diagnosed with or are at risk of being afflicted with an angiogenic eye disorder. Generally, the methods of the present invention demonstrate efficacy within 104 weeks of the initiation of the treatment regimen (with the initial dose administered at "week 0"), *e.g.*, by the end of week 16, by the end of week 24, by the end of week 32, by the end of week 40, by the end of week 48, by the end of week 56, etc. In the context of methods for treating angiogenic eye disorders such as AMD, CRVO, and DME, "efficacy" means that, from the initiation of treatment, the

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patient exhibits a loss of 15 or fewer letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart. In certain embodiments, "efficacy" means a gain of one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or more) letters on the ETDRS chart from the time of initiation of treatment.

EXAMPLES

[0034] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

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

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

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

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

[0037] This study was a double-masked, randomized study of 3 doses (0.5, 2, and 4 mg) of VEGFT tested at 4-week and/or 12-week dosing intervals. There were 5 treatment arms in this study, as follows: 1) 0.5 mg every 4 weeks, 2) 0.5 mg every 12 weeks, 3) 2 mg every 4 weeks, 4) 2 mg every 12 weeks and 5) 4 mg every 12 weeks. Subjects were dosed at a fixed interval for the first 12 weeks, after which they were evaluated every 4 weeks for 9 months, during which additional doses were administered based on pre-specified criteria. All subjects were then followed for one year after their last dose of VEGFT. Preliminary data from a pre-planned interim analysis indicated that VEGFT met its primary endpoint of a statistically significant reduction in retinal thickness after 12 weeks compared with baseline (all groups combined, decrease of 135μ, $p < 0.0001$). Mean change from baseline in visual acuity, a key secondary endpoint of the study, also demonstrated statistically significant improvement (all groups combined, increase of 5.9 letters, $p < 0.0001$). Moreover, patients in the dose groups that received only a single dose, on average, demonstrated a decrease in excess retinal thickness ($p < 0.0001$) and an increase in visual acuity ($p = 0.012$) at 12 weeks. There were no drug-related serious adverse events, and treatment with the VEGF antagonists was generally well-tolerated. The most common adverse events were those typically associated with intravitreal injections.

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

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

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

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

A. Objectives, Hypotheses and Endpoints

[0040] Two parallel Phase III clinical trials were carried out to investigate the use of VEGFT to treat patients with the neovascular form of age-related macular degeneration (Study 1 and Study 2). The primary objective of these studies was to assess the efficacy of IVT administered VEGFT compared to ranibizumab (Lucentis®, Genentech, Inc.), in a non-inferiority paradigm, in preventing moderate vision loss in subjects with all subtypes of neovascular AMD.

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

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

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

B. Study Design

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

[0045] The study duration for each subject was scheduled to be 96 weeks plus the recruitment period. For the first 52 weeks (Year 1), subjects received an IVT or sham injection in the study eye every 4 weeks. (No sham injections were given at Week 52). During the second year of the study, subjects will be evaluated every 4 weeks and will receive IVT injection of study drug at intervals determined by specific dosing criteria, but at least every 12 weeks. (During the second year of the study, sham injections will not be given.) During this period, injections may be given as frequently

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as every 4 weeks, but no less frequently than every 12 weeks, according to the following criteria: (i) increase in central retinal thickness of ≥100 μm compared to the lowest previous value as measured by optical coherence tomography (OCT); or (ii) a loss from the best previous letter score of at least 5 ETDRS letters in conjunction with recurrent fluid as indicated by OCT; or (iii) new or persistent fluid as indicated by OCT; or (iv) new onset classic neovascularization, or new or persistent leak on fluorescein angiography (FA); or (v) new macular hemorrhage; or (vi) 12 weeks have elapsed since the previous injection. According to the present protocol, subjects must receive an injection at least every 12 weeks.

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

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

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

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

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

[0051] Exclusion criteria for both studies were as follows: 1. Any prior ocular (in the study eye) or systemic treatment or surgery for neovascular AMD except dietary supplements or vitamins. 2. Any prior or concomitant therapy with another investigational agent to treat neovascular AMD in the study eye, except dietary supplements or vitamins. 3. Prior treatment with anti-VEGF agents as

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

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the subject beyond what is to be expected from standard procedures of intraocular injection, or which otherwise may interfere with the injection procedure or with evaluation of efficacy or safety. 30. History of other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that might affect interpretation of the results of the study or render the subject at high risk for treatment complications. 31. Participation as a subject in any clinical study within the 12 weeks prior to Day 1. 32. Any systemic or ocular treatment with an investigational agent in the past 3 months prior to Day 1. 33. The use of long acting steroids, either systemically or intraocularly, in the 6 months prior to day 1. 34. Any history of allergy to povidone iodine. 35. Known serious allergy to the fluorescein sodium for injection in angiography. 36. Presence of any contraindications indicated in the FDA Approved label for ranibizumab (Lucentis®). 37. Females who were pregnant, breastfeeding, or of childbearing potential, unwilling to practice adequate contraception throughout the study. Adequate contraceptive measures include oral contraceptives (stable use for 2 or more cycles prior to screening); IUD; Depo-Provera®; Norplant® System implants; bilateral tubal ligation; vasectomy; condom or diaphragm plus either contraceptive sponge, foam or jelly.

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

[0053] The study procedures are summarized as follows:

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

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

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[0056] Fundus Photography and Fluorescein Angiography (FA): The anatomical state of the retinal vasculature of the study eye was evaluated by funduscopic examination, fundus photography and FA. At the Screen Visit (Visit 1) funduscopic examination, fundus photography and FA were captured and transmitted for both eyes. Fundus and angiographic images were sent to an independent reading center where images were read by masked readers. The reading center confirmed subject eligibility based on angiographic criteria prior to randomization. All FAs and fundus photographs were archived at the site as part of the source documentation. Photographers were required to be certified by the reading center to ensure consistency and quality in image acquisition. Adequate efforts were made to ensure that all photographers at the site remain masked to treatment assignment.

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

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

[0059]

C. Results Summary (52 Week Data)

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

Table 1

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

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

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

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

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

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

Table 2

	n	Mean change in visual acuity at week 24 versus baseline	Mean change in visual acuity at week 52 versus baseline

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		(letters)	(letters)
Laser	44	2.5	-1.3
VEGFT 0.5 mg monthly (0.5Q4)	44	8.6**	11.0**
VEGFT 2 mg monthly (2Q4)	44	11.4**	13.1**
VEGFT 2 mg every 8 weeks[a] (2Q8)	42	8.5**	9.7**
VEGFT 2 mg as needed[a] (PRN)	45	10.3**	12.0**

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

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

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

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

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

[0067] At Week 24, 56.1% of VEGFT-treated patients gained ≥15 ETDRS letters from baseline vs 12.3% of sham-treated patients ($P<0.0001$). Similarly, at Week 52, 55.3% of VEGFT-treated patients gained ≥15 letters vs 30.1% of sham-treated patients ($P<0.01$). At Week 52, VEGFT-treated patients gained a mean of 16.2 letters vs 3.8 letters for sham-treated patients ($P<0.001$). Mean number of injections was 2.7 for VEGFT-treated patients vs 3.9 for sham-treated patients. Mean change in central retinal thickness was -413.0 µm for VEGFT-treated patients vs -381.8 µm

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for sham-treated patients. The proportion of patients with ocular neovascularization at Week 24 were 0% for VEGFT-treated patients and 6.8% for sham-treated patients, respectively; at Week 52 after receiving VEGFT PRN, proportions were 0% and 6.8% for VEGFT-treated and sham-treated. At Week 24, the mean change from baseline in the VFQ-25 total score was 7.2 vs 0.7 for the VEGFT-treated and sham-treated groups; at Week 52, the scores were 7.5 vs 5.1 for the VEGFT-treated and sham-treated groups.

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

Example 7: Dosing Regimens

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

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

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

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

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

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

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

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

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

[0078] VEGFT 2 mg (0.5 mL) administered by intravitreal injection once every 4 weeks for the first 16 weeks, followed by 2 mg (0.05 mL) via intravitreal injection on a less frequent basis based on visual and/or anatomical outcomes (as assessed by a physician or other qualified medical professional).

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

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

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

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

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

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

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

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

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

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

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

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

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

SEQUENCES

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

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTCAC
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CACTGTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCATAATCTGG
GACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGCTTCTGACCTGT
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AAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGGACCGTCAGTCTTCCTCT
TCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTG
GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGT

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GCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG
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GAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC
AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

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

MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLK
KFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGI
ELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRS
DQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

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

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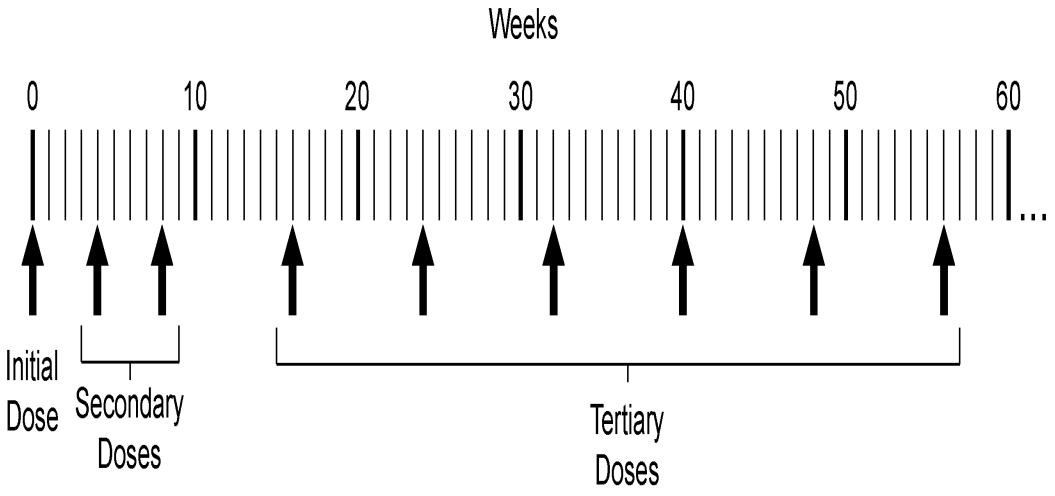

Weeks
0
10
20
30
40
50
60
...
Initial Dose
Secondary Doses
Tertiary Doses

Figure 1

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT International application number 13/940,370

filed on July 12, 2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: YANCOPOULOS, GEORGE D. Date (Optional): 10/20/13

Signature: /

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/AIA/01 form for each additional inventor.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Privacy Act Statement

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

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

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.
10.

REMARKS UNDER 37 CFR § 1.115

Formal Matters

Claims 21-49 are pending after entry of the amendments set forth herein.

Claims 1-20 are canceled without prejudice.

Claims 21-49 are added.

Support for new claims 21-49 can be found in originally pending now canceled claims 1-20, and throughout the specification.

The specification has been amended to update the cross-reference to related application section.

No new matter has been added.

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

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

The Applicants wish 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 Applicants wish to bring to the Examiner's attention U.S. Patent Application No. 14/972,560, filed December 17, 2015 which issued on June 6, 2018 as U.S. Patent No. 9,669,069.

The Applicants wish 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. 10,130,691..

The Applicants wish to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/055,847, filed August 6, 2018 for which no actions have been mailed.

The Applicants wish to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/159,282, filed October 12, 2018 for which a non-final Office Action was mailed April 3, 2019.

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.

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CONCLUSION

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

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-008CIPCON5.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 29 April 2019

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
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AMENDMENTS TO THE CLAIMS

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

21. (**New**) A method for treating age related macular degeneration in a patient, comprising administering, to said patient, by intravitreal injection, 2 mg aflibercept approximately every 4 weeks for the first 3 months, followed by 2 mg aflibercept approximately once every 8 weeks or once every 2 months.

22. (**New**) The method of claim 21, wherein the age-related macular degeneration is neovascular (wet).

23. (**New**) The method of claim 21, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

24. (**New**) The method of claim 23, wherein the age-related macular degeneration is neovascular (wet).

25. (**New**) The method of claim 22 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

26. (**New**) The method of claim 25 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

27. (**New**) The method of claim 22 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

28. (**New**) The method of claim 27 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

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29. (**New**) A method for treating diabetic macular edema in a patient, comprising administering, to said patient, by intravitreal injection, 2 mg aflibercept approximately every 4 weeks for the first 5 injections followed by 2 mg aflibercept approximately once every 8 weeks or once every 2 months.

30. (**New**) The method of claim 29, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

31. (**New**) The method of claim 29, further comprising, after 20 weeks, administering, via intravitreal injection, 2 mg of aflibercept once every 4 weeks.

32. (**New**) The method of claim 29 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

33. (**New**) The method of claim 32 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

34. (**New**) The method of claim 29 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

35. (**New**) The method of claim 34 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

36. (**New**) A method for treating diabetic retinopathy in a patient, comprising administering, to said patient, by intravitreal injection, 2 mg aflibercept approximately every 4 weeks for the first 5 injections followed by 2 mg aflibercept approximately once every 8 weeks or 2 months.

37. (**New**) The method of claim 36, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

38. (**New**) The method of claim 36, further comprising after 20 weeks, administering via intravitreal injection 2 mg of aflibercept once every 4 weeks

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39. (**New**) The method of claim 36 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

40. (**New**) The method of claim 37 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

41. (**New**) The method of claim 36 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

42. (**New**) The method of claim 41 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

43. (**New**) A method for treating diabetic retinopathy in a patient with diabetic macular edema, comprising administering, to said patient, by intravitreal injection, 2 mg aflibercept approximately every 4 weeks for the first 5 injections followed by 2 mg aflibercept approximately once every 8 weeks or 2 months.

44. (**New**) The method of claim 43, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

45. (**New**) The method of claim 43, further comprising after 20 weeks, administering via intravitreal injection 2 mg of aflibercept once every 4 weeks

46. (**New**) The method of claim 43 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

47. (**New**) The method of claim 46 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

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48. (**New**) The method of claim 43 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

49. (**New**) The method of claim 48 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

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DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 16397267 Document Date: 04/29/2019

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217-9197 (toll free).
Reviewer: Wheat Jr, Scott (ASRC)
Timestamp: [year=2019; month=5; day=3; hr=11; min=24; sec=27; ms=123; ]
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Validated By CRFValidator v 1.0.5

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SEQUENCE LISTING

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

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<141> 2019-04-29

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
16/397,267	04/29/2019	1629	3080	REGN-008CIPCON5	29	4

CONFIRMATION NO. 8135

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

FILING RECEIPT

OC000000108021427

Date Mailed: 05/09/2019

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

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Inventor(s)

George D. YANCOPOULOS, Yorktown Heights, NY;

Applicant(s)

REGENERON PHARMACEUTICALS, INC., Tarrytown, NY

Assignment For Published Patent Application

REGENERON PHARMACEUTICALS, INC., Tarrytown, NY

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 16/159,282 10/12/2018
which is a CON of 15/471,506 03/28/2017 PAT 10130681
which is a CON of 14/972,560 12/17/2015 PAT 9669069
which is a CON of 13/940,370 07/12/2013 PAT 9254338
which is a CIP of PCT/US2012/020855 01/11/2012
which claims benefit of 61/432,245 01/13/2011
and claims benefit of 61/434,836 01/21/2011
and claims benefit of 61/561,957 11/21/2011

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

Mylan Exhibit 1017
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Page 61

Samsung Bioepis Exhibit 1017, pg. 61

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

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

If Required, Foreign Filing License Granted: 05/08/2019

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/397,267**

Projected Publication Date: 08/15/2019

Non-Publication Request: No

Early Publication Request: No

Title

USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

Preliminary Class

514

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

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Page 62

Samsung Bioepis Exhibit 1017, pg. 62

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/397,267

APPLICATION AS FILED - PART I

	(Column 1)	(Column 2)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE($)	FEE($)		RATE($)	FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	300
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	660
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	760
TOTAL CLAIMS (37 CFR 1.16(i))	29 minus 20 =	* 9			OR	x 100 =	900
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1				x 460 =	460
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	3080

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	YANCOPOULOS, GEORGE D.
				Art Unit	N/A
				Examiner Name	Jon McClelland Lockard
Sheet	1	of	5	Attorney Docket Number	REGN-008CIPCON5

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	7303746	2007-12-04	Wiegand	
	2	7303748	2007-12-04	Wiegand	
	3	7306799	2007-12-11	Wiegand	
	4	7396664	2008-07-08	Daly et al.	
	5	9254338	2016-02-09	Yancopoulos	
	6	9669069	2017-06-06	Yancopoulos	
	7	10130681	2018-11-20	Yancopoulos	

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	20050163798	2005-07-28	Papadopoulos et al.	
	2	20050260203	2005-11-24	Wiegand et al.	
	3	20060058234	2006-03-16	Daly et al.	
	4	20060172944	2006-08-03	Wiegand et al.	
	5	20070190058	2007-08-16	Shams	
	6	20030171320	2003-09-11	Guyer	

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No.	Foreign Document Number Country Code-Number-Kind Code (*if known*)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	1	WO 2006/047325	2006-03-04	Genentech, Inc.		
	2	WO 2000/75319	2000-12-14	Regeneron Pharmaceuitcals, Inc.		
	3	WO 2007/022101 A2	2007-02-22	Regeneron Pharmaceuticals, Inc.		
	4	WO 2008/063932	2008-05-29	Genentech, Inc.		
	5	JP 2010-509369	2010-03-25	Genentech, Inc.	See WO 2008/063932 for English Equivalent	
	6	WO 2012/097019	2012-07-19	Regeneron Pharmaceuticals, Inc.		

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T
	1	ANONYMOUS "Lucentis (rangibizymab injection) Intravitreal Injection" pp. 103 (June 2006)	
	2	BROWNING et al. "Aflibercept for age-related macular degeneration: a game-changer or quiet addition?" American Journal of Ophthalmology, Vol. 154(2):222-226 (08/01/2012)	

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	YANCOPOULOS, GEORGE D.
				Art Unit	N/A
				Examiner Name	Jon McClelland Lockard
Sheet	2	of	5	Attorney Docket Number	REGN-008CIPCON5

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
	3	CAMPOCHIARO et al. "Ranibizumab for Macular Edema Due to Retinal Vein Occlusions Implication of VEGF as a Critical Stimulator" Molecular Therapy, 16(4):791-799 (2008)	
	4	Information from ClinicalTrials.gov archive View of NCT00637377 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD) (VIEW 2)" *ClinicalTrials.gov*. Web. 2010-11-30.	
	5	CENTER FOR DRUG EVALUATION AND RESEARCH APPLICATION NUMBER: 21-756 MEDICAL REVIEW(S) (December 17, 2004) <URL:https://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-756_Macugen_medr.pdf>	
	6	CENTER FOR DRUG EVALUATION AND RESEARCH BLA APPLICATION NUMBER: 125156 MEDICAL REVIEW, (June 2006) <URL:https://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/125156s000_Lucentis_MedR.pdf>	
	7	CAO, "A Subretinal Matrigel Rat Choroidal Neovascularization (CNV) Model and Inhibition of CNV and Associated Inflammation and Fibrosis by VEGF Trap" Investigative Ophthalmology & Visual Science, 51(11):6009- 6017 (11/2010)	
	8	CHARLES, Steve (Guest Lecturer) "VEGF Trap Has Positive DME Data" Tenth Annual Retina Fellows Forum Jan 29 and 30, Chicago, Article Date 03/01/2010	
	9	DIXON et al., "VEGF Trap-Eye for the treatment of neobascular age-related macular degeneration" Expert Opin. Investig. Drugs (2009) 18 (10): 1-8.	
	10	DO et al., "An exploratory study of the safety, tolerability and bioactivity of a single intravitreal injection of vascular endothelial growth factor Trap-Eye in patients with diabetic macular oedema" Br J Opthamol. 93(2):144-1449 (February 2009)	
	11	DO et al., "The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema" Opthamology 118(9):1819-1826 (September 2011)	
	12	EICHTEN, "Rapid decrease in tumor perfusion following VEGF blockade predicts long-term tumor growth inhibition in preclinical tumor models" Angiogenesis, 16:429-441 (2013)	
	13	THE EYETECH STUDY GROUP, "Anti-Vascular Endothelial Growth Factor Therapy for Subfoveal Choroidal Neovascularization Secondary to Age-related Macular Degeneration" American Academy of Ophthamology, 110(5):979-986 (May 2003)	
	14	HEIERet al., " rhuFab V2 (anti-VEGF Antibody) for Treatment of Exudative AMD" Symposium 8:Experimental and Emerging Treatments for Choroidal Neovascularization, 10 pp (2002)	
	15	HEIER et al., "RhuFab V2 in Wet AMD - 6 Month Continued Improvement Following Multiple Intravitreal Injections" Invest Ophthalmol Vis Sci, 44:E-Abstract 972 (2003)	
	16	HEIER et al., "Intravitreal Aflibercept (VEGF Trap-Eye) in Wet Age-related macular Degeneration," Ophthalmology, 119:2537-2548 (2012)	
	17	HO, "VEGF Trap-Eye in Wet AMD - CLEAR-IT 2: One-Year OCT and FA Outcomes" CLEAR-IT 2 Study Group, pp 1-24 (09/28/2008)	
	18	HOLASH, "VEGF-Trap: A VEGF blocker with potent antitumor effects" PNAS 99(17)11393-11398 (8/20/2002)	
	19	HOLASH, "Inhibitors of growth factor receptors, signaling pathways and angiogenesis as therapeutic molecular agents." Cancer Metastasis 25:243-252 (2006)	

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	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	YANCOPOULOS, GEORGE D.
				Art Unit	N/A
				Examiner Name	Jon McClelland Lockard
Sheet	3	of	5	Attorney Docket Number	REGN-008CIPCON5

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
	20	Information from ClinicalTrials.gov archive on the VIEW 2 study (NCT00637377) "VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD (VIEW 2)" version available and updated on 17 March 2008.	
	21	Information from ClinicalTrials.gov archive on the view of NCT00509795 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD)" (12-01-2009)	
	22	Information from ClinicalTrials.gov archive on the view of NCT00789477 "DME and VEGF Trap-Eye: Investigation of Clinical Impact" (11-18-2010)	
	23	Information from ClinicalTrials.gov archive on the view of NCT00509795 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Wet Age-Related Macular Degeneration (AMD)" (01-07-2011)	
	24	KARIA, Niral, "Retinal vein occlusion: pathophysiology and treatment options" Clinical Ophthalmology, 4:809-816 (2010)	
	25	KUO, "Comparative evaluation of the antitumor activity of antiangiogenic proteins delivered by gene transfer" PNAS 98(8):4605-4610 (04/10/2001)	
	26	KRZYSTOLIK et al., "Prevention of Experimental Choroidal NEovascularization With Intravitreal Anti-Vascular Endothelial Growth Factor Antibody Fragment" Arch Ophthamol., 120:338-346 (Mar. 2002)	
	27	Lucentis Label Title, 7 pages, 30/06/2010 **[Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR]**	
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	36	PAI et al., "Current concepts in intravitreal drug therapy for diabetic retinopathy" Saudi Journal of Opthamology 24(4):143-149 (June 30, 2010)	

Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	YANCOPOULOS, GEORGE D.
				Art Unit	N/A
				Examiner Name	Jon McClelland Lockard
Sheet	4	of	5	Attorney Docket Number	REGN-008CIPCON5

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
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	39	Regeneron, Press release "Regeneron Reports First Quarter 2008 Financial and Operating Results", May 1, 2008.	
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	42	Regeneron Press Release "Regeneron and Bayer Report Positive Results for VEGF Trap-Eye in Phase 3 Study in Central Retinal Vein Occlusion (CRVO) and in Phase 2 Study in Diabetic Macular Edema (DME)" December 20, 2010	
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	52	THOMAS REUTERS INTEGRITY "VEGF Trap-Eye final phase II results in age-related macular degeneration presented at 2008 Retina Society Meeting" (September 28, 2008)	
	53	THURSTON, "Vascular endothelial growth factor and other signaling pathways in developmental and pathologic angiogenesis." International Journal of Hematology 80:7-20 (2004)	

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	YANCOPOULOS, GEORGE D.
				Art Unit	N/A
				Examiner Name	Jon McClelland Lockard
Sheet	5	of	5	Attorney Docket Number	REGN-008CIPCON5

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
	54	WACHSBERGER, "VEGF trap in combination with radiotherapy improves tumor control in u87 glioblastoma." Int. J. Radiation Oncology Biol Phys. 67(5):1526-1537 (2007)	
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	59	Vascular Endothelial Growth Factor Trap‐ Eye Investigation of Efficacy and Safety in Central Retinal Vein Occlusion title, 8 pages, 11/12/2009, US **[Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR]**	

Examiner Signature		Date Considered	

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

Electronic Acknowledgement Receipt	
EFS ID:	36351485
Application Number:	16397267
International Application Number:	
Confirmation Number:	8135
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-008CIPCON5
Receipt Date:	19-JUN-2019
Filing Date:	29-APR-2019
Time Stamp:	17:50:43
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	Transmittal Letter	REGN-008CIPCON5_2019-06-19_IDS_trans.pdf	52048 ea0b2da55b1503c70404a22bd20ee1ac4584afb0	no	2

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIPCON5_2019-06-19_IDS_SB08A.pdf	69369 1ed84d7d4d1bb583a8b744bf7e12778f135b446e	no	5
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
		Total Files Size (in bytes):	121417		

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

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronically Filed 6/19/2019

INFORMATION DISCLOSURE STATEMENT	Attorney Docket No.	REGN-008CIPCON5
	Confirmation No.	8135
	First Named Inventor	George D. Yancopoulos
	Application Number	16/397,267
	Filing Date	April 29, 2019
	Group Art Unit	
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.

The Examiner is requested to make the documents listed on the enclosed PTO/SB/08A of record in this application. Applicants would appreciate the Examiner initialing and returning the initialed copy of form PTO/SB/08A, indicating the documents cited therein have been considered and made of record herein.

All of the references identified herein were disclosed in parent application serial number 16/159,282, and as such, copies thereof are not included pursuant to the provisions of 37 CFR § 1.98(d).

Statements

☒ **No statement**

☐ **PTA Statement under 37 CFR § 1.704(d)(1):** Each item of information contained in the information disclosure statement filed herewith:

(i) Was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office, and this communication was not received by any individual designated in § 1.56(c) more than thirty days prior to the filing of the information disclosure statement; or

(ii) Is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office, and this communication was not received by any individual designated in § 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

☐ **IDS Statement under 37 CFR § 1.97(e)(1):** Each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement; or

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

Fees

☒ No fee is believed to be due.

☐ The appropriate fee set forth in 37 C.F.R. §1.17(p) accompanies this information disclosure statement.

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-008CIPCON5.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: June 19, 2019

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

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PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/397,267	Filing Date 04/29/2019	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $100 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $460 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	08/14/2019	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 33	Minus	** 29	= 4		x $100 =	400
	Independent (37 CFR 1.16(h))	* 5	Minus	*** 4	= 1		x $460 =	460
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	860

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

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

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronically Filed

SECOND PRELIMINARY AMENDMENT Under CFR 1.115 Address to: Mail Stop Patent Application Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450		
	Attorney Docket No.	REGN-008CIPCON5
	Confirmation No.	8135
	First Named Inventor	YANCOPOULOS, GEORGE D.
	Application Number	16/397,267
	Filing Date	April 29, 2019
	Group Art Unit	1647
	Examiner Name	LOCKARD, Jon McClelland
	Title: *"Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders"*	

Sir:

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

AMENDMENTS TO THE CLAIMS

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

21. (Previously Presented) A method for treating age related macular degeneration in a patient, comprising administering, to said patient, by intravitreal injection, 2 mg aflibercept approximately every 4 weeks for the first 3 months, followed by 2 mg aflibercept approximately once every 8 weeks or once every 2 months.

22. (Previously Presented) The method of claim 21, wherein the age-related macular degeneration is neovascular (wet).

23. (Previously Presented) The method of claim 21, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

24. (Previously Presented) The method of claim 23, wherein the age-related macular degeneration is neovascular (wet).

25. (Previously Presented) The method of claim 22 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

26. (Previously Presented) The method of claim 25 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

27. (Previously Presented) The method of claim 22 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

28. (Previously Presented) The method of claim 27 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

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29. (Previously Presented) A method for treating diabetic macular edema in a patient, comprising administering, to said patient, by intravitreal injection, 2 mg aflibercept approximately every 4 weeks for the first 5 injections followed by 2 mg aflibercept approximately once every 8 weeks or once every 2 months.

30. (Previously Presented) The method of claim 29, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

31. (Previously Presented) The method of claim 29, further comprising, after 20 weeks, administering, via intravitreal injection, 2 mg of aflibercept once every 4 weeks.

32. (Previously Presented) The method of claim 29 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

33. (Previously Presented) The method of claim 32 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

34. (Previously Presented) The method of claim 29 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

35. (Previously Presented) The method of claim 34 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

36. (Previously Presented) A method for treating diabetic retinopathy in a patient, comprising administering, to said patient, by intravitreal injection, 2 mg aflibercept approximately every 4 weeks for the first 5 injections followed by 2 mg aflibercept approximately once every 8 weeks or 2 months.

37. (Previously Presented) The method of claim 36, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

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38. (Previously Presented) The method of claim 36, further comprising after 20 weeks, administering via intravitreal injection 2 mg of aflibercept once every 4 weeks

39. (Previously Presented) The method of claim 36 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

40. (Previously Presented) The method of claim 37 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

41. (Previously Presented) The method of claim 36 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

42. (Previously Presented) The method of claim 41 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

43. (Previously Presented) A method for treating diabetic retinopathy in a patient with diabetic macular edema, comprising administering, to said patient, by intravitreal injection, 2 mg aflibercept approximately every 4 weeks for the first 5 injections followed by 2 mg aflibercept approximately once every 8 weeks or 2 months.

44. (Previously Presented) The method of claim 43, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

45. (Previously Presented) The method of claim 43, further comprising after 20 weeks, administering via intravitreal injection 2 mg of aflibercept once every 4 weeks

46. (Previously Presented) The method of claim 43 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

47. (Previously Presented) The method of claim 46 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

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48. (Previously Presented) The method of claim 43 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

49. (Previously Presented) The method of claim 48 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

50. (**New**) A method for treating an angiogenic eye disorder in a patient, said method comprising sequentially administering to the patient

a single initial dose of a VEGF antagonist, followed by

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

one or more tertiary doses of the VEGF antagonist;

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

wherein each tertiary dose is administered 8 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 and Ig domain 3 of a second VEGF receptor, and a multimerizing component.

51. (**New**) The method of claim 50 wherein the first VEGF receptor is Flt1 and the second VEGF receptor is Flk1.

52. (**New**) The method of claim 50 wherein the VEGF antagonist is aflibercept.

53. (**New**) The method of claim 51, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

54. (**New**) The method of claim 53, wherein the intraocular administration is intravitreal administration.

55. (**New**) The method of claim 54, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

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56. (**New**) The method of claim 55, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

57. (**New**) The method of claim 55, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

58. (**New**) The method of claim 51, 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.

59. (**New**) The method of claim 51 wherein the angiogenic eye disorder is age related macular degeneration.

60. (**New**) The method of claim 51 wherein the angiogenic eye disorder is diabetic retinopathy.

61. (**New**) The method of claim 51, wherein the angiogenic eye disorder is diabetic macular edema.

62. (**New**) The method of claim 59 wherein all doses of VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

63. (**New**) The method of claim 59 wherein all doses of VEGF antagonist comprise 2.0 mg of the VEGF antagonist.

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REMARKS UNDER 37 CFR § 1.115

Formal Matters

Claims 21-63 are pending after entry of the amendments set forth herein.

Original claims 1-20 were previously canceled without prejudice.

Claims 21-49 were previously added in the Preliminary Amendment filed April 29, 2019.

Claims 50-63 are added here.

Support for new claims 50-63 as well as previously added claims 21-49 can be found in originally pending now canceled claims 1-20, and throughout the specification.

No new matter has been added.

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

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

The Applicants wish 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 Applicants wish to bring to the Examiner's attention U.S. Patent Application No. 14/972,560, filed December 17, 2015 which issued on June 6, 2018 as U.S. Patent No. 9,669,069.

The Applicants wish 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. 10,130,691..

The Applicants wish to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/055,847, filed August 6, 2018 for which no actions have been mailed.

The Applicants wish to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/159,282, filed October 12, 2018 for which a non-final Office Action was mailed April 3, 2019.

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.

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Samsung Bioepis Exhibit 1017, pg. 82

CONCLUSION

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

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-008CIPCON5.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 14 August 2019

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

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Mylan Exhibit 1017
Mylan v. Regeneron, IPR2022-01226
Page 83

Samsung Bioepis Exhibit 1017, pg. 83

Electronic Patent Application Fee Transmittal	
Application Number:	16397267
Filing Date:	29-Apr-2019
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Filer:	Karl Bozicevic/Savanna Fuentes
Attorney Docket Number:	REGN-008CIPCON5
Filed as Large Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
CLAIMS IN EXCESS OF 20	1202	4	100	400
INDEPENDENT CLAIMS IN EXCESS OF 3	1201	1	460	460
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**860**

Electronic Acknowledgement Receipt	
EFS ID:	36873462
Application Number:	16397267
International Application Number:	
Confirmation Number:	8135
Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
Filer:	Karl Bozicevic/Savanna Fuentes
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON5
Receipt Date:	14-AUG-2019
Filing Date:	29-APR-2019
Time Stamp:	16:57:54
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$860
RAM confirmation Number	E20198DG58088162
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		REGN-008CIPCON5_2019-08-14_supp_pre_amend.pdf	50102 92cd1b790a43367a7c992bfc6946afcc520b8ed1	yes	8

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Preliminary Amendment	1	1
	Claims	2	6
	Applicant Arguments/Remarks Made in an Amendment	7	8

Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	32341 284bd4278c910f6b381685ce97aca740c847a3d9	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	82443		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/397,267	04/29/2019	George D. YANCOPOULOS	REGN-008CIPCON5

CONFIRMATION NO. 8135

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

PUBLICATION NOTICE

OC000000110424420

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

Publication No.US-2019-0247463-A1
Publication Date:08/15/2019

NOTICE OF PUBLICATION OF APPLICATION

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

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

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

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

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

Mylan Exhibit 1017
Mylan v. Regeneron, IPR2022-01226
Page 89

Samsung Bioepis Exhibit 1017, pg. 89

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	Yancopoulos, George D.
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	1	of	3	Attorney Docket Number	REGN-008CIPCON5

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	**2008/0220004**	2008-09-11	Wiegand et al.	
	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	BARBAZETTO, "DOSING REGIMEN AND THE FREQUENCY OF MACULAR HEMORRHAGES IN NEOVASCULAR AGE-RELATED MACULAR DEGENERATION TREATED WITH RANIBIZUMAB." Retina, 30:9, 1376-85, 2010	
	2	Bayer Investor News, "Bayer and Regeneron Start additional Phase 3 Study for VEGF Trap=Eye in Wet Age-related Macular Degeneration." May 8, 2008	
	3	BOYER, "A Phase IIIb Study to Evaluate the Safety of Ranibizumab in Subjects with Neovascular Age-related Macular Degeneration." Opthalmology, 116:9, 1731-39, September 2009.	
	4	BROWN, "Ranibizumab versus Verteporfin for Neovascular Age-Related Macular Degeneration." N Engl J Med 355:14, 1432-44, October 5, 2006	
	5	BROWN, "Primary Endpoint Results of a Phase II Study of Vascular Endothelial Growth Factor Trap-Eye in Wet Age-related Macular Degeneration." Ophthalmology, 118:6, 1089-97, June 2011.	
	6	BROWN, "Long-term Outcomes of Ranibizumab Therapy for Diabetic Macular Edema: The 36-Month Results from Two phase III Trials." Ophthalmology, 2013.	
	7	CAMPOCHIARO, "Ranibizumab for Macular Edema following Branch Retinal Vein Occlusion." Ophthalmology, 2010.	
	8	CAMPOCHIARO, "Sustained Benefits from Ranibizumab for Macular Edema following Central Retinal Vein Occlusion: Twelve-Month Outcomes of a phase III Study." Ophthalmology, 188:10, 2041-49, October 2011.	
	9	CSAKY, "Safety Implications of Vascular Endothelial Growh Factor Blockade for Subjects Receiving Intravitreal Anti-Vascular Endothelial Growth Factor Therapies." Am. J. Ophthalmology, 148:5, 647-56, November 2009.	

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	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	Yancopoulos, George D.
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	2	of	3	Attorney Docket Number	REGN-008CIPCON5

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	DO, "One-Year Outcomes of the DA VINCI Study of VEGF Trap-Eye in Eyes with Diabetic Macular Edema." Ophthalmology, 2012.	
	11	ENGELBERT, "The 'Treat and Extend' Dosing Regimen of Intravitreal Anti-Vascular Endothelial Growth Factor Therapy for Neovascular Age-Related Macular Degeneration." Ophthalmology Management, June 2010, available at http://www.visioncareprofessional.com/emails/amdupdate/index.asp?issue=42	
	12	ENGELBERT, "LONG-TERM FOLLOW-UP FOR TYPE 1 (SUBRETINAL PIGMENT EPITHELIUM) NEOVASCULARIZATION USING A MODIFIED 'TREAT AND EXTEND' DOSING REGIMENT OF INTRAVITREAL ANTIVASCULAR ENDOTHELIAL GROWTH FACTOR THERAPY." Retina, 30:9, 1368-75, 2010	
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	14	Eylea®, Highlights of Prescribing Information, Revised 08/2018.	
	15	FUNG, "An Optical Coherence Tomography-Guided, Variable Dosing Regiment with Intravitreal Ranibizumab (Lucentis) for Neovascular Age-related Macular Degeneration." Am J Ophthalmology 143:4, 566-83, April 2007	
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	18	GOMEZ-MANZANO, "VEGF Trap induces antiglioma effect at different stages of disease." Society for Neuro-Oncology, December 2008.	
	19	GRAGOUDAS, "Pegaptanib for Neovascular Age-Related Macular Degeneration." N Engl J Med 351:27, 2805-16, December 30, 2004	
	20	HEIER, "Intravitreal Aflibercept for Diabetic Macular Edema." Ophthalmology, 2016.	
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	26	LEVINE, "MACULAR HEMORRHAGE IN NEOVASCULAR AGE-RELATED MACULAR DEGENERATION AFTER STABILIZATION WITH ANTIANGIOGENIC THERAPY." Retina, 29:8, 1074-79, 2009.	

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	Yancopoulos, George D.
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	3	of	3	Attorney Docket Number	REGN-008CIPCON5

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
	27	MARGOLIS, "HEMORRHAGIC RECURRENCE OF NEOVASCULAR AGE-RELATED MACULAR DEGENERATION NOT PREDICTED BY SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY." Retinal Cases & Brief Reports, 4:1, 2010	
	28	MASSIN, "Safety and Efficacy of Ranibizumab in Diabetic Macular Edema (RESOLVE Study*)." Diabetes Care, 33:11, 2399-405, November 2010.	
	29	MITCHELL, "The RESTORE Study, Ranibizumab Monotherapy or Combined with Laser versus Laser Monotherapy for Diabetic Macular Edema." Ophthalmology, 188:4, 615-25, April 2011.	
	30	NGUYEN, "Ranibizumab for Diabetic Macular Edema, Results from 2 Phase III Randomized Trials: RISE and RIDE." Ophthalmology, 119:4, 789-801, April 2012.	
	31	Regeneron Press Release, "Bayer and Regeneron Dose First Patient in Second Phase 3 Study for VEGF Trap-Eye in Wet Age-Related Macular Degeneration." May 8, 2008	
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	39	YANCOPOULOS, "Vascular-specific growth factors and blood vessel formation." Nature 407:242-48, September 14, 2000.	
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	41	YUNG, "moving Toward the Next Steps in Angiogenesis Therapy?" Society for Neuro-Oncology, 10:939, 2008	

Examiner Signature		Date Considered	

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

Electronic Acknowledgement Receipt	
EFS ID:	37202823
Application Number:	16397267
International Application Number:	
Confirmation Number:	8135
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-008CIPCON5
Receipt Date:	18-SEP-2019
Filing Date:	29-APR-2019
Time Stamp:	15:32:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	REGN-008CIPCON5_2019-09-18_IDS_trans.pdf	52094 a69133c25dca9be2f4b37b6b9eb6aacb2fd2b76	no	2

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	REGN-008CIPCON5_2019-09-18_IDS_SB08A.pdf	49068 bbbe2ea0a56368f82ff36d9a1f1689ece59d960e	no	3
Warnings:					
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New Applications Under 35 U.S.C. 111

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

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

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New International Application Filed with the USPTO as a Receiving Office

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Electronically Filed 9/18/2019

INFORMATION DISCLOSURE STATEMENT	Attorney Docket No.	REGN-008CIPCON5
	Confirmation No.	8135
	First Named Inventor	George D. Yancopoulos
	Application Number	16/397,267
	Filing Date	April 29, 2019
	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.

The Examiner is requested to make the documents listed on the enclosed PTO/SB/08A of record in this application. Applicants would appreciate the Examiner initialing and returning the initialed copy of form PTO/SB/08A, indicating the documents cited therein have been considered and made of record herein.

All of the references identified herein were disclosed in parent application serial number 16/159,282, and as such, copies thereof are not included pursuant to the provisions of 37 CFR § 1.98(d).

Statements

☒ **No statement**

☐ **PTA Statement under 37 CFR § 1.704(d)(1):** Each item of information contained in the information disclosure statement filed herewith:

(i) Was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office, and this communication was not received by any individual designated in § 1.56(c) more than thirty days prior to the filing of the information disclosure statement; or

(ii) Is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office, and this communication was not received by any individual designated in § 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

☐ **IDS Statement under 37 CFR § 1.97(e)(1):** Each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement; or

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

Fees

☒ No fee is believed to be due.

☐ The appropriate fee set forth in 37 C.F.R. §1.17(p) accompanies this information disclosure statement.

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-008CIPCON5.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: September 18, 2019

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

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Document Description: Power of Attorney

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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date
16/397,267	April 29, 2019

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 96387

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

☑ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

Regeneron Pharmaceuticals, Inc.

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	/Frank R. Cottingham/	Date (Optional)	September 6, 2019
Name	Frank R. Cottingham		
Title	Executive Director, Assistant General Counsel, Patents, Regeneron Pharmaceuticals, Inc.		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/96 (08-12)
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STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Regeneron Pharmaceuticals, Inc.

Application No./Patent No.: 16/397,267 Filed/Issue Date: April 29, 2019

Titled: Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders

Regeneron Pharmaceuticals, Inc., a Corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☑ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

   ☐ The extent (by percentage) of its ownership interest is _____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☑ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 050278, Frame 0613, or for which a copy thereof is attached.

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

   1. From: _____ To: _____

      The document was recorded in the United States Patent and Trademark Office at

      Reel _____, Frame _____, or for which a copy thereof is attached.

   2. From: _____ To: _____

      The document was recorded in the United States Patent and Trademark Office at

      Reel _____, Frame _____, or for which a copy thereof is attached.

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☑ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Karl Bozicevic, Reg. No. 28,807/	2019/09/27
Signature	Date
Karl Bozicevic	28,807
Printed or Typed Name	Title or Registration 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.

Electronic Acknowledgement Receipt	
EFS ID:	37284164
Application Number:	16397267
International Application Number:	
Confirmation Number:	8135
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/Halle Jarman
Filer Authorized By:	Karl Bozicevic
Attorney Docket Number:	REGN-008CIPCON5
Receipt Date:	27-SEP-2019
Filing Date:	29-APR-2019
Time Stamp:	18:37:04
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	0725US06_POA.pdf	169021 4f8368c019dbee50cae97a4f05b90c9acf7fd883	no	1

Warnings:

Information:					
2	Power of Attorney	REGN-008CIPCON5_aia0096.pdf	118186 685a2d201bf52f3aa05d8a008e168775302 9dae0	no	3
Warnings:					
Information:					
		Total Files Size (in bytes):	287207		

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
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/397,267	04/29/2019	George D. YANCOPOULOS	REGN-008CIPCON5

CONFIRMATION NO. 8135

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

POA ACCEPTANCE LETTER

OC000000111575443

Date Mailed: 10/02/2019

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 09/27/2019.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ltaba/

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Electronically Filed

SECOND PRELIMINARY AMENDMENT Under CFR 1.115 Address to: Mail Stop Patent Application Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450		
	Attorney Docket No.	REGN-008CIPCON5
	Confirmation No.	8135
	First Named Inventor	YANCOPOULOS, GEORGE D.
	Application Number	16/397,267
	Filing Date	April 29, 2019
	Group Art Unit	1647
	Examiner Name	LOCKARD, Jon McClelland
	Title: *"Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders"*	

Sir:

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

Electronic Acknowledgement Receipt	
EFS ID:	38221376
Application Number:	16397267
International Application Number:	
Confirmation Number:	8135
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-008CIPCON5
Receipt Date:	07-JAN-2020
Filing Date:	29-APR-2019
Time Stamp:	12:27:41
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		0725-US06_REGN-008CIPCON5_2020-01-07_third_supp_prelim_amend.pdf	52805 131002db171e4170e2a8116e4bfce7cf64bb5876	yes	8

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Applicant Arguments/Remarks Made in an Amendment	7	8
	Claims	2	6
	Preliminary Amendment	1	1
Warnings:			
Information:			
	Total Files Size (in bytes):	52805	

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.

REMARKS UNDER 37 CFR § 1.115

Formal Matters

Claims 21-63 are pending after entry of the amendments set forth herein.

Original claims 1-20 were previously canceled without prejudice.

Claims 21, 29, 36, 38, 43, 45 and 50 have been amended to more particularly point out and distinctly claim the invention. Support for these amendments can be found throughout the specification.

No new matter has been added.

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

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

The Applicants wish 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 Applicants wish to bring to the Examiner's attention U.S. Patent Application No. 14/972,560, filed December 17, 2015 which issued on June 6, 2018 as U.S. Patent No. 9,669,069.

The Applicants wish 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. 10,130,691.

The Applicants wish to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/055,847, filed August 6, 2018 for which a non-final Office Action was mailed on December 10, 2019.

The Applicants wish to bring to the Examiner's attention co-pending U.S. Patent Application No. 16/159,282, filed October 12, 2018 for which a non-final Office Action was mailed October 1, 2019.

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.

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CONCLUSION

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

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-008CIPCON5.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 7 January 2020

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

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

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AMENDMENTS TO THE CLAIMS

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

21. (**Currently amended**) A method for treating age related macular degeneration in a patient, comprising **intravitreally** administering, to said patient, ~~**by intravitreal injection,**~~ **an effective amount of aflibercept which is** 2 mg ~~**aflibercept**~~ approximately every 4 weeks for the first 3 months, followed by 2 mg ~~**aflibercept**~~ approximately once every 8 weeks or once every 2 months.

22. (Previously Presented) The method of claim 21, wherein the age-related macular degeneration is neovascular (wet).

23. (Previously Presented) The method of claim 21, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

24. (Previously Presented) The method of claim 23, wherein the age-related macular degeneration is neovascular (wet).

25. (Previously Presented) The method of claim 22 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

26. (Previously Presented) The method of claim 25 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

27. (Previously Presented) The method of claim 22 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

28. (Previously Presented) The method of claim 27 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

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29. (**Currently amended**) A method for treating diabetic macular edema in a patient, comprising **intravitreally** administering, to said patient~~, by intravitreal injection~~, **an effective amount of aflibercept which is** 2 mg ~~aflibercept~~ approximately every 4 weeks for the first 5 injections followed by 2 mg ~~aflibercept~~ approximately once every 8 weeks or once every 2 months.

30. (Previously Presented) The method of claim 29, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

31. (Previously Presented) The method of claim 29, further comprising, after 20 weeks, administering, via intravitreal injection, 2 mg of aflibercept once every 4 weeks.

32. (Previously Presented) The method of claim 29 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

33. (Previously Presented) The method of claim 32 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

34. (Previously Presented) The method of claim 29 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

35. (Previously Presented) The method of claim 34 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

36. (**Currently amended**) A method for treating diabetic retinopathy in a patient, comprising **intravitreally** administering, to said patient, ~~by intravitreal injection,~~ **an effective amount of aflibercept which is** 2 mg ~~aflibercept~~ approximately every 4 weeks for the first 5 injections followed by 2 mg ~~aflibercept~~ approximately once every 8 weeks or 2 months.

37. (Previously Presented) The method of claim 36, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

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38. (**Currently amended**) The method of claim 36, further comprising**,** after 20 weeks, administering**,** via intravitreal injection**,** 2 mg of aflibercept once every 4 weeks**.**

39. (Previously Presented) The method of claim 36 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

40. (Previously Presented) The method of claim 37 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

41. (Previously Presented) The method of claim 36 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

42. (Previously Presented) The method of claim 41 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

43. (**Currently amended**) A method for treating diabetic retinopathy in a patient with diabetic macular edema, comprising **intravitreally** administering, to said patient, ~~**by intravitreal injection,**~~ **an effective amount of aflibercept which is** 2 mg ~~**aflibercept**~~ approximately every 4 weeks for the first 5 injections followed by 2 mg ~~**aflibercept**~~ approximately once every 8 weeks or 2 months.

44. (Previously Presented) The method of claim 43, wherein approximately every 4 weeks comprises approximately every 28 days or approximately monthly.

45. (**Currently amended**) The method of claim 43, further comprising**,** after 20 weeks, administering**,** via intravitreal injection**,** 2 mg of aflibercept once every 4 weeks**.**

46. (Previously Presented) The method of claim 43 wherein the patient loses less than 15 letters of Best Corrected Visual Acuity (BCVA) score.

47. (Previously Presented) The method of claim 46 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

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48. (Previously Presented) The method of claim 43 wherein the patient gains at least 15 letters of Best Corrected Visual Acuity (BCVA) score.

49. (Previously Presented) The method of claim 48 wherein Best Corrected Visual Acuity (BCVA) is measured by Early Treatment Diabetic Retinopathy Study (ETDRS) letter score.

50. (**Currently Amended**) A method for treating an angiogenic eye disorder in a patient, said method comprising ~~**sequentially**~~ administering to the patient **an effective sequential dosing regimen of** 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 8 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 and Ig domain 3 of a second VEGF receptor, and a multimerizing component.

51. (Previously Presented) The method of claim 50 wherein the first VEGF receptor is Flt1 and the second VEGF receptor is Flk1.

52. (Previously Presented) The method of claim 50 wherein the VEGF antagonist is aflibercept.

53. (Previously Presented) The method of claim 51, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

54. (Previously Presented) The method of claim 53, wherein the intraocular administration is intravitreal administration.

55. (Previously Presented) The method of claim 54, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

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56. (Previously Presented) The method of claim 55, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

57. (Previously Presented) The method of claim 55, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

58. (Previously Presented) The method of claim 51, 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.

59. (Previously Presented) The method of claim 51 wherein the angiogenic eye disorder is age related macular degeneration.

60. (Previously Presented) The method of claim 51 wherein the angiogenic eye disorder is diabetic retinopathy.

61. (Previously Presented) The method of claim 51, wherein the angiogenic eye disorder is diabetic macular edema.

62. (Previously Presented) The method of claim 59 wherein all doses of VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

63. (Previously Presented) The method of claim 59 wherein all doses of VEGF antagonist comprise 2.0 mg of the VEGF antagonist.

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Samsung Bioepis Exhibit 1017, pg. 113

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/397,267	Filing Date 04/29/2019	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $100 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $460 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	08/14/2019	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 43	Minus	** 29	= 14		x $100 =	1400
	Independent (37 CFR 1.16(h))	* 5	Minus	*** 4	= 1		x $460 =	460
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	1860

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	01/07/2020	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 43	Minus	** 43	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 5	Minus	*** 5	= 0		x $460 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/LAVINIA D JOHNSON/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Sale Accounting Date:01/14/2020

Sale Item Reference Number	Effective Date
16397267	08/14/2019

Document Number	Fee Code	Fee Code Description	Amount Paid	Payment Method
I20201DK35179217	1202	CLAIMS IN EXCESS OF 20	$1,000.00	Deposit Account

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	George D. Yancopoulos
				Art Unit	
				Examiner Name	
Sheet	1	of	2	Attorney Docket Number	REGN-008CIPCON5

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	16/055,847 – Third Party Submissions dated May 1, 2019	
	2	16/159,282 – Third Party Submissions dated May 31, 2019	
	3	BROWN, "Long-term Outcomes of Ranibizumab Therapy for Diabetic Macular Edema: The 36-Month Results from Two phase III Trials." Ophthalmology, 120(10):2013-22 (October 2013)	
	4	CAMPOCHIARO, "Ranibizumab for Macular Edema following Branch Retinal Vein Occlusion: six-month primary end point results of a phase III study." Ophthalmology, 117(6):1102-1112 (June 2010)	
	5	DIXON et al., "VEGF Trap-Eye for the treatment of neovascular age-related macular degeneration" Expert Opin. Investig. Drugs, 18(10):1573-1580 (2009)	
	6	DO, "One-Year Outcomes of the DA VINCI Study of VEGF Trap-Eye in Eyes with Diabetic Macular Edema." Ophthalmology, 119(8):1658-65 (2012)	
	7	ENGELBERT, "The 'Treat and Extend' Dosing Regimen of Intravitreal Anti-Vascular Endothelial Growth Factor Therapy for Neovascular Age-Related Macular Degeneration." Ophthalmology Management, Issue 42, (June 2010) available at http://www.visioncareprofessional.com/emails/amdupdate/index.asp?issue=42	
	8	GOMEZ-MANZANO, "VEGF Trap induces antiglioma effect at different stages of disease." Neuro-Oncology, 10:940-945 (December 2008)	
	9	GUTIERREZ et al., "Intravitreal bevacizumab (Avastin) in the treatment of macular edema secondary to retinal vein occlusion" Clin. Ophthalmol., 2(4):787,791 (2008)	
	10	HEIER, "Intravitreal Aflibercept for Diabetic Macular Edema: 148-Week Results from the VISTA and VIVID Studies." Ophthalmology, 123(11):2376-2385 (November 2016)	

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	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	George D. Yancopoulos
				Art Unit	
				Examiner Name	
Sheet	2	of	2	Attorney Docket Number	REGN-008CIPCON5

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
	11	Information from ClinicalTrials.gov archive on the view of NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 7 pages, first posted 11/13/2009; results first posted 11/22/2012; last update posted 11/3/14; printed 12/4/19 (https://clinicaltrials.gov/ct2/show/study/NCT01012973) (NOTE: May correspond to "Vascular Endothelial Growth Factor Trap‐ Eye Investigation of Efficacy and Safety in Central Retinal Vein Occlusion title, 8 pages, 11/12/2009, US **[Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR]"** which was cited in the Third Party Observations dated 05/01/19)	
	12	KAISER, "Vascular endothelial growth factor Trap-Eye for diabetic macular oedema." Br. J. Ophthalmol, 93(2):135-36 (February 2009)	
	13	MARGOLIS, "Hemorrhagic Recurrence Of Neovascular Age-Related Macular Degeneration Not Predicted By Spectral Domain Optical Coherence Tomography." Retinal Cases & Brief Reports, 4:1-4 (2010)	
	14	NICHOLS, EARL R., "AAO: Ranibizumab (rhuRab) May Improve Vision in Age-Related Macular Degeneration" Doctor's Guide Global Edition, www.pslgroup.com/dg/23f2aa.htm, pp. 1-2 (November 24, 2003)	
	15	SCHMIDT-ERFURTH, "Efficacy and Safety of Monthly versus Quarterly Ranibizumab Treatment in Neovascular Age-related Macular Degeneration: The EXCIE Study" Ophthalmology, 118(5)831-839 (2010)	
	16	SCHNICHELS, "Comparative toxicity and proliferation testing of aflibercept, bevacizumab and ranibizumab on different ocular cells." Br. J. Ophthalmol., 97:917-923 (2013)	
	17	SIMO AND HERNANDEZ, "Advances in Medical Treatment of Diabetic Retinopathy" Diabetes Care, 32(8):1556-1562 (August 2009)	
	18	SPAIDE, "Ranibizumab According to Need: A Treatment for Age-related Macular Degeneration." Am J Ophthalmology, 143(4):679-680 (April 2007)	
	19	Vascular Endothelial Growth Factor Trap‐ Eye Investigation of Efficacy and Safety in Central Retinal Vein Occlusion title, 8 pages, 11/12/2009, US **[Cited in Third Party Observations filed in parent application USSN 16/055,847 for which a copy is unavailable on PAIR] NOTE:** May correspond to **"**Information from ClinicalTrials.gov archive on the view of NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 7 pages, first posted 11/13/2009; results first posted 11/22/2012; last update posted 11/3/14; printed 12/4/19 (https://clinicaltrials.gov/ct2/show/study/NCT01012973)" cited by the Examiner in the Office Action dated 12/10/19 in USSN 16/055,847	
	20	YANCOPOULOS, "Clinical Application of Therapies Targeting VEGF." Cell 143:13-16 (October 1, 2010)	

Examiner Signature		Date Considered	

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

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/055,847	08/06/2018	George D. Yancopoulos	REGN-008CIPCON3	3451

96387 7590 05/01/2019

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
05/01/2019	ELECTRONIC

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

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

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docket@bozpat.com

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APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR/ PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
16/055,847	08/06/2018	Yancopoulos, George D.	REGN-008CIPCON3

		EXAMINER	
Regeneron - Bozicevic, Field & Francis 201 REDWOOD SHORES PARKWAY SUITE 200 REDWOOD CITY, CA 94065		MARIANNE C SEIDEL	
		ART UNIT	PAPER
		1600	20190429

DATE MAILED: _____

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

Commissioner for Patents

The third-party submission under 37 CFR 1.290 filed on 4/21/19 and 4/27/19 for the instant application has been determined to be compliant with 35 U.S.C. 122(e) and 37 CFR 1.290 and is being entered in the application. Please allow a few days for the submission to be visible in the Patent Application Information Retrieval (PAIR) system.

/MARIANNE C SEIDEL/ Quality Assurance Specialist, Art Unit 1600	

PTO-90C (Rev.04-03)

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290
CONCISE DESCRIPTION OF RELEVANCE

Application Number	16055847

U.S. PATENTS

Cite No	Patent Number	Concise Description of Relevance

U.S. PATENT APPLICATION PUBLICATION

Cite No	Publication Number	Concise Description of Relevance

FOREIGN PATENT DOCUMENTS		
CiteNo	**Foreign Document Number**	**Concise Description of Relevance**

NON-PATENT PUBLICATIONS		
Cite No	**Reference**	**Concise Description of Relevance**
1	Vascular Endothelial Growth Factor Trap‐Eye Investigation of Efficacy and Safety in Central Retinal Vein Occlusion title, 8 pages, 11/12/2009, US	See Attached

2	Niral Karia Author, Retinal vein occlusion: pathophysiology and treatment options title, 8 pages, 07/31/2010, Clinical Ophthalmology publisher	See Attached

3	RAFAEL Author, Advances in the Medical Treatment of Diabetic Retinopathy, 7 pages, 08/31/2009, DIABETES CARE publisher	See Attached

PTO/SB/429(08-12)
Approved for use through 07/31/2015. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	16055847

U.S. PATENTS				
Cite No	Patent Number	Kind Code[1]	Issue Date (YYYY-MM-DD)	First Named Inventor

U.S. PATENT APPLICATION PUBLICATIONS				
Cite No	Publication Number	Kind Code[1]	Publication Date (YYYY-MM-DD)	First Named Inventor

FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS						
Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[1]	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T[5]
						☐

NON-PATENT PUBLICATIONS (e.g., journal article, Office action)			
Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T[5]	E[6]

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	16055847

1	RAFAEL Author, Advances in the Medical Treatment of Diabetic Retinopathy, 7 pages, 08/31/2009, DIABETES CARE publisher	☐	☒
2	Niral Karia Author, Retinal vein occlusion: pathophysiology and treatment options title, 8 pages, 07/31/2010, Clinical Ophthalmology publisher	☐	☒
3	Vascular Endothelial Growth Factor Trap‐Eye Investigation of Efficacy and Safety in Central Retinal Vein Occlusion title, 8 pages, 11/12/2009, US	☐	☒

STATEMENTS

The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56.

This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

EFS Web 2.1.17

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	16055847

☐ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.			
☒ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.			
☐ This resubmission is being made responsive to a notification of non-compliance issued for an earlier filed third-party submission. The corrections in this resubmission are limited to addressing the non-compliance. As such, the party making this resubmission: (1) requests that the Office apply the previously-paid fee set forth in 37 CFR 1.290(f), or (2) states that no fee is required to accompany this resubmission as the undersigned is again making the fee exemption statement set forth in 37 CFR 1.290(g).			
Signature	/Joe Reynolds/		
Name/Print	Joe	Registration Number (if applicable)	
Examiner Signature		Date Considered	

*EXAMINER: Signature indicates all documents listed above have been considered,except for citations through which a line is drawn. Draw line through citation if not considered. Include a copy of this form with next communication to applicant. 1. If known, enter kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 2. Enter the country or patent office that issued the document, by two-letter code under WIPO standard ST.3. See MPEP 1851. 3. For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 4. If known, enter the kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 . See MPEP 901.04(a). 5. Check mark indicates translation attached. 6. Check mark indicates evidence of publication attached.

Mylan Exhibit 1017
Mylan v. Regeneron, IPR2022-01226
Page 126

Samsung Bioepis Exhibit 1017, pg. 126

Concise Description of Relevance

The following references are being submitted:

P1: Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO); web site: https://clinicaltrials.gov/ct2/history/NCT01012973?V_1=View#StudyPageTop; Published on Nov 12, 2009;

P2: Niral Karia, Retinal vein occlusion: pathophysiology and treatment options. Clinical Ophthalmology 2010:4 809–816, Published on Jul, 2010;

P3: RAFAEL SIM ´O and CRISTINA HERN´ANDEZ, Advances in the Medical Treatment of Diabetic Retinopathy. DIABETES CARE, VOLUME 32, NUMBER 8, AUGUST 2009, page 1559, Published on Aug, 2009.

As the claim chart shows below, **P1** discloses study description of clinical trial NCT01012973, which aims to determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion. The only difference is that Claim 21 uses "2 mg aflibercept" instead of "VEGF Trap-Eye" used in P1;

P2 discloses that Retinal vein occlusion (RVO) is the most common retinal vascular disease after diabetic retinopathy. Depending on the area of retinal venous drainage effectively occluded it is broadly classified as either central retinal vein occlusion (CRVO), hemispheric retinal vein occlusion (HRVO), or branch retinal vein occlusion (BRVO). (*see* page 809 introduction section first two sentences);

P3 discloses that aflibercept also known as a VEGF Trap-Eye because its ability to block VEGF proteins, is a fusion protein comprised of segments of the extracellular domains of human VEGF receptor 1 and 2 fused to the constant region of human IgG. (*see* page 1559 right column third paragraph);

Besides, as it is mentioned in the present invention, the VEGF antagonist comprises one or more VEGF receptor-based chimeric molecule(s), (also referred to herein as a "VEGF-Trap" or "VEGFT"). An exemplary VEGF antagonist that can be used in the context of the present invention is a multimeric VEGF-binding protein comprising two or more VEGF receptor-based chimeric molecules referred to herein as "VEGFR1R2-FcΔC1(a)" or "aflibercept." (*see* paragraph [0008]);

	U.S. Application No. 16/055,847	Concise Description of Relevance
Claim #	Claim Elements	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)
21	A method for treating macular edema following retinal vein occlusion in a human subject comprising administering 2 mg aflibercept to the subject	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion. [Study Description]
	by intravitreal injection	Intravitreal injection. [Assigned Interventions]
	once every 4 weeks.	Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks [Assigned Interventions]

Electronic Acknowledgement Receipt	
EFS ID:	35851526
Application Number:	16055847
International Application Number:	
Confirmation Number:	3451
Title of Invention:	
First Named Inventor/Applicant Name:	
Correspondence Address:	- - - - - - - - - - -
Filer:	Joe Reynolds
Filer Authorized By:	
Attorney Docket Number:	
Receipt Date:	27-APR-2019
Filing Date:	
Time Stamp:	21:09:41
Application Type:	

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	Concise Description of Relevance	Concise-description-generated.pdf	32661 846d65f66190992370cec1261748f68cf4cc673f	no	4
Warnings:					
Information:					
2	Third-Party Submission Under 37 CFR 1.290	Third-party-preissuance-submission.pdf	44466 e6a9d076030d0aca379d98a73373d704e9b2afc6	no	3
Warnings:					
Information:					
3	Request for Notification of Non-compliant Third-Party Submission	Third-party-notification-request.pdf	20027 458cf58b9516e443d102fb4934eaadf17bbf64ec	no	1
Warnings:					
Information:					
4	Concise Description of Relevance	Concise-description.pdf	116758 f737b13f87e153c3f9661833e3265158eedb8133	no	2
Warnings:					
Information:					
5	Evidence of Publication	Evidence-pub-3.pdf	86747 a92ecf8c1f3e7d8e4825700b9d68497d1d6be612	no	7
Warnings:					
Information:					
6	Evidence of Publication	Evidence-pub-2.pdf	162155 73c3a794cd2d69eeb692c5a0add3e4473a05a979	no	8
Warnings:					
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		Total Files Size (in bytes):	462814		

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.

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290
CONCISE DESCRIPTION OF RELEVANCE

Application Number	16055847

U.S. PATENTS

Cite No	Patent Number	Concise Description of Relevance

U.S. PATENT APPLICATION PUBLICATION

Cite No	Publication Number	Concise Description of Relevance

FOREIGN PATENT DOCUMENTS		
CiteNo	**Foreign Document Number**	**Concise Description of Relevance**

NON-PATENT PUBLICATIONS		
Cite No	**Reference**	**Concise Description of Relevance**
1	Peter AUTHER, Ranibizumab for Macular Edema Due to Retinal Vein Occlusions Implication of VEGF as a Critical Stimulator title, 9 pages , 30/08/2008	See Attached

2	Lucentis Label title ,7 pages, 30/06/2010	See Attached

PTO/SB/429(08-12)
Approved for use through 07/31/2015. OMB 0651-0031
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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	16055847

U.S. PATENTS				
Cite No	Patent Number	Kind Code[1]	Issue Date (YYYY-MM-DD)	First Named Inventor

U.S. PATENT APPLICATION PUBLICATIONS				
Cite No	Publication Number	Kind Code[1]	Publication Date (YYYY-MM-DD)	First Named Inventor

FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS						
Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[1]	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T[5]
						☐

NON-PATENT PUBLICATIONS (e.g., journal article, Office action)			
Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T[5]	E[6]

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	16055847

1	Lucentis Label title ,7 pages, 30/06/2010	☐	☒
2	Peter AUTHER, Ranibizumab for Macular Edema Due to Retinal Vein Occlusions Implication of VEGF as a Critical Stimulator title, 9 pages , 30/08/2008	☐	☒

STATEMENTS

The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56.

This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

☐ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.

☒ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.

☐ This resubmission is being made responsive to a notification of non-compliance issued for an earlier filed third-party submission. The corrections in this resubmission are limited to addressing the non-compliance. As such, the party making this resubmission: (1) requests that the Office apply the previously-paid fee set forth in 37 CFR 1.290(f), or (2) states that no fee is required to accompany this resubmission as the undersigned is again making the fee exemption statement set forth in 37 CFR 1.290(g).

Mylan Exhibit 1017
Mylan v. Regeneron, IPR2022-01226
Page 136

Samsung Bioepis Exhibit 1017, pg. 136

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	16055847

Signature	/Elizabeth Thompson/		
Name/Print	Elizabeth	Registration Number (if applicable)	
Examiner Signature		Date Considered	

*EXAMINER: Signature indicates all documents listed above have been considered,except for citations through which a line is drawn. Draw line through citation if not considered. Include a copy of this form with next communication to applicant. 1. If known, enter kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 2. Enter the country or patent office that issued the document, by two-letter code under WIPO standard ST.3. See MPEP 1851. 3. For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 4. If known, enter the kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 . See MPEP 901.04(a). 5. Check mark indicates translation attached. 6. Check mark indicates evidence of publication attached.

Electronic Acknowledgement Receipt	
EFS ID:	35783629
Application Number:	16055847
International Application Number:	
Confirmation Number:	3451
Title of Invention:	
First Named Inventor/Applicant Name:	
Correspondence Address:	- - - - - - - - - - -
Filer:	Elizabeth Thompson
Filer Authorized By:	
Attorney Docket Number:	
Receipt Date:	21-APR-2019
Filing Date:	
Time Stamp:	21:29:30
Application Type:	

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	Concise Description of Relevance	Concise-description-generated.pdf	30185 1fd17ba5e73b9f30386d2803b71e9bc73fd08cb4	no	3
Warnings:					
Information:					
2	Third-Party Submission Under 37 CFR 1.290	Third-party-preissuance-submission.pdf	43151 79d1d8f0f8ebf66c58ca2108d13dcc72cab805d0	no	3
Warnings:					
Information:					
3	Request for Notification of Non-compliant Third-Party Submission	Third-party-notification-request.pdf	20074 40d5d061fee7b35a11a9fd0caf93f69559afcb7d	no	1
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		Total Files Size (in bytes):	93410		

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New International Application Filed with the USPTO as a Receiving 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 05/31/2019

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
05/31/2019	ELECTRONIC

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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):

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Mylan Exhibit 1017
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Page 140

Samsung Bioepis Exhibit 1017, pg. 140

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

		EXAMINER	
Regeneron - Bozicevic, Field & Francis 201 REDWOOD SHORES PARKWAY SUITE 200 REDWOOD CITY, CA 94065		MARIANNE C SEIDEL	
		ART UNIT	PAPER
		1600	20190529

DATE MAILED: _____

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Commissioner for Patents

The third-party submission under 37 CFR 1.290 filed on 5/28/19 for the instant application has been determined to be compliant with 35 U.S.C. 122(e) and 37 CFR 1.290 and is being entered in the application. Please allow a few days for the submission to be visible in the Patent Application Information Retrieval (PAIR) system.

/MARIANNE C SEIDEL/
Quality Assurance Specialist, Art Unit 1600

PTO-90C (Rev.04-03)

Concise Description of Relevance

VEGF Trap-Eye for the Treatment of Neovascular Age-Related Macular Degeneration

James A. Dixon et al. *Expert Opin. Investig. Drugs* (**2009**) 18(10):1573-1580 (hereafter "Dixon")

Dixon is prior art under pre-AIA §102(b) because it published more than one year before the earliest effective filing date (January 13, 2011) of U.S. Application No. 16/159,282 (hereafter "the '282 application).

U.S. Application No. 16/159,282	Relevant disclosure in Dixon
21. A method for treating an angiogenic eye disorder in a patient, said method comprising	**Dixon** is related to clinical trial results of treating neovascular age-related macular degeneration (wet AMD) with VEGF Trap-eye. Wet AMD is an angiogenic eye disorder. *See e.g.*, ***Background/p. 1573***, disclosing that aflibercept (VEGF Trap-eye) is a promising therapy for AMD by interrupting angiogenesis. *See also* the definition of "angiogenic eye disorder" in the '282 application (para. [0004]).
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;	Phase II trial ***(§2.6.2, p. 1576***) teaches that patients with CNV (subfoveal choroidal neovascularization), a subtype of AMD, received monthly dose of 0.5mg or 2mg for 12 weeks (0, 4, 8, 12) followed by treatment of the same dose on a PRN basis. Thus, DIXON teaches AMD patients being treated by (1) a **single initial dose** of 0.5mg or 2mg at **week 0**, followed by **3 secondary doses** in 4-week intervals (*i.e.*, on **week 4, 8 and 12 after the initial dose**); followed by **tertiary doses** on **PRN basis**. **Dixon** further teaches criteria for re-dosing, including basis on visual (ETDRS letters) or anatomical (retinal thickness by OCT).
wherein each secondary dose is administered **4 weeks after the immediately preceding dose**; and	
wherein each **tertiary dose is administered on an as-needed/pro re nata (PRN) basis, based on visual and/or anatomical outcomes as assessed by a physician or other qualified medical professional**;	
wherein the **VEGF antagonist** is a receptor-based chimeric molecule comprising an immunoglobin-like (Ig) domain 2 of a first VEGF receptor and Ig domain 3 of a second VEGF	VEGF Trap-eye is a VEGF antagonist having key binding domains of human VEGFR-1 and -2 combined with a human

receptor, and a multimerizing component.	IgG Fc fragment. (***§2.2, p 1575***)
22. The method of claim 21, wherein the VEGF antagonist is **aflibercept**.	VEGF Trap-eye is chemically identical to aflibercept. (***§2.3, p 1575***)
23. The method of claim 22, wherein all doses of the VEGF antagonist are administered to the patient by **intraocular** administration.	Phase II trial ***(§2.6.2, p. 1576***) teaches AMD patients received VEGF Trap-eye in one eye (*i.e.*, intraocular administration). **Dixon** teaches intravitreal injection throughout.
24. The method of claim 23, wherein the intraocular administration is **intravitreal** administration.	Phase II trial ***(§2.6.2, p. 1576***) teaches AMD patients received VEGF Trap-eye in one eye (*i.e.*, intraocular administration). **Dixon** teaches intravitreal injection throughout.
25. The method of claim 24, wherein all doses of the VEGF antagonist comprise from about **0.5 mg** to about **2 mg** of the VEGF antagonist.	Phase II trial ***(§2.6.2, p. 1576***) teaches AMD patients received VEGF Trap-eye in 0.5mg or 2mg per dose.
28. The method of claim 27, 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.	AMD is an angiogenic eye disorder.
29. The method of claim 28 wherein the angiogenic eye disorder is age related macular degeneration.	AMD is an angiogenic eye disorder.
30. The method of claim 28 wherein the angiogenic eye disorder is diabetic retinopathy.	VEGF Trap-Eye was used to treat diabetic retinopathy (***§2.6.1, p1575, the third paragraph***).
31. The method of claim 28, wherein the angiogenic eye disorder is diabetic macular edema.	VEGF Trap-Eye was used to treat diabetic macular edema ***(§2.6.1, p1575, the fourth paragraph***).

Concise Description of Relevance

Enrollment Completed in Regeneron and Bayer HealthCare Two Phase 3 Studies of VEGF Trap-Eye in Neovascular Age-Related Macular Degeneration (Wet AMD)

Regeneron Press Release dated **September 14, 2009** (hereafter "**Regeneron**")

https://newsroom.regeneron.com/static-files/6611111b9-3da3-459b-b0da-c74f00ab4b32

Regeneron is prior art under pre-AIA §102(b) because it published more than one year before the earliest effective filing date (January 13, 2011) of U.S. Application No. 16/159,282 (hereafter "the '282 application."

U.S. Application No. 16/159,282	**Relevant disclosure in Regeneron (with evidentiary support from Dixon)**
32. A method for treating an angiogenic eye disorder in a patient, said method comprising	**Regeneron** discloses the VIEW Program, which included Phase 3 studies for treating neovascular age-related macular degeneration (wet AMD). Wet AMD is a known angiogenic eye disorder. *See also* definition of "angiogenic eye disorder" in the '282 application (para. [0004]).
sequentially administering to the patient a **single initial dose** of a VEGF antagonist, followed by **one or more secondary doses** of the VEGF antagonist, followed by **one or more tertiary doses** of the VEGF antagonist;	The VIEW Program teaches patients with wet AMD are to receive regular doses of 0.5mg or 2mg VEGF Trap-eye at 4-week intervals in the first year, followed by continued treatment for another year on a flexible, criteria-based extended PRN regimen with a dose administered ***at least every 12 weeks***, but not more often than every four weeks. Thus, **Regeneron** teaches AMD patients being treated by (1) a ***single initial dose*** of 0.5mg or 2mg, followed by (2) ***secondary doses at 4-week intervals*** for a year, followed by (3) treatment for another year based on a flexible schedule, which would include ***at least one tertiary dose at 12-week*** from the immediately preceding dose. *See also*, Example 4 of the '282 application, which tracks closely to the VIEW program. Example 4 provides the written support for the dosing regimen recited in claim 32.
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-	VEGF Trap-eye is an anti-VEGF agent, a soluble

based chimeric molecule comprising an immunoglobin-like (Ig) domain 2 of a first VEGF receptor and Ig domain 3 of a second VEGF receptor, and a multimerizing component.	VEGF receptor fusion protein that binds all forms of VEGF-A along with the related placental growth factor (PIGF). *See* "About VEGF Trap-Eye; *see also* evidentiary support in **Dixon**.
33. The method of claim 22*, wherein the VEGF antagonist is **aflibercept**. * *It appears that claim 32 was intended.*	VEGF Trap-eye is chemically identical to aflibercept. *See also* evidentiary support in **Dixon (*§2.3, p 1575*)**.
34. The method of claim 23*, wherein all doses of the VEGF antagonist are administered to the patient by **intraocular** administration. * *It appears that claim 33 was intended.*	The VIEW program evaluates the effect of VEGF Trap-eye "on maintaining and improving vision when dosed as an ***intravitreal*** injection…" *See* the first paragraph. Intravitreal injection is a type of intraocular administration.
35. The method of claim 23*, wherein the intraocular administration is **intravitreal** administration. * *It appears that claim 33 was intended.*	The VIEW program evaluates the effect of VEGF Trap-eye "on maintaining and improving vision when dosed as an ***intravitreal*** injection…" *See* the first paragraph of page 1.
36. The method of claim 25*, wherein all doses of the VEGF antagonist comprise from about **0.5 mg** to about **2 mg** of the VEGF antagonist. * *It appears that claim 35 was intended.*	The VIEW program evaluates the effect of VEGF Trap-eye of two dosing strengths (0.5mg and 2mg) administered on regular schedules. *See* paragraph 1 and paragraph 4 on page 1.
37. The method of claim 36, wherein all doses of the VEGF antagonist comprise **0.5 mg** of the VEGF antagonist.	
38. The method of claim 36, wherein all doses of the VEGF antagonist comprise **2 mg** of the VEGF antagonist.	
39. 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	**Regeneron** teaches that VEGF Trap-eye is used for treating eye diseases, including wet AMD, diabetic macular edema (DME), and Central Retinal Vein Occlusion (CRVO). *See* the second paragraph on page 1.

corneal neovascularization.	
40. The method of claim 39 wherein the angiogenic eye disorder is age related macular degeneration.	**Regeneron** teaches treating AMD throughout.
41. The method of claim 39 wherein the angiogenic eye disorder is diabetic retinopathy.	**Regeneron** teaches that the primary endpoint is evaluated using the standard Early Treatment Diabetic Retinopathy Study (ETDRS) chart for visual acuity, suggesting that an effective therapy for treating AMD could also be used to treat diabetic retinopathy.
42. The method of claim 39, wherein the angiogenic eye disorder is diabetic macular edema.	**Regeneron** teaches VEGF Trap-eye is also for treatment of diabetic macular edema. *See* the second paragraph on page 2.

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290
CONCISE DESCRIPTION OF RELEVANCE

Application Number	16159282

U.S. PATENTS

Cite No	Patent Number	Concise Description of Relevance

U.S. PATENT APPLICATION PUBLICATION

Cite No	Publication Number	Concise Description of Relevance

FOREIGN PATENT DOCUMENTS		
CiteNo	**Foreign Document Number**	**Concise Description of Relevance**

NON-PATENT PUBLICATIONS		
Cite No	**Reference**	**Concise Description of Relevance**
1	Regeneron Press Release dated September 14, 2009	See Attached

2	James A. Dixon et al., Expert Opin. Investig. Drugs (2009) 18(10):1573-1580	See Attached

PTO/SB/429(08-12)
Approved for use through 07/31/2015. OMB 0651-0031
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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	16159282

U.S. PATENTS				
Cite No	Patent Number	Kind Code[1]	Issue Date (YYYY-MM-DD)	First Named Inventor

U.S. PATENT APPLICATION PUBLICATIONS				
Cite No	Publication Number	Kind Code[1]	Publication Date (YYYY-MM-DD)	First Named Inventor

FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS						
Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[1]	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T[5]
						☐

NON-PATENT PUBLICATIONS (e.g., journal article, Office action)			
Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T[5]	E[6]

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	16159282

1	James A. Dixon et al., Expert Opin. Investig. Drugs (2009) 18(10):1573-1580	☐	☐
2	Regeneron Press Release dated September 14, 2009	☐	☐

STATEMENTS

The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56.

This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

☐ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.

☒ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.

☐ This resubmission is being made responsive to a notification of non-compliance issued for an earlier filed third-party submission. The corrections in this resubmission are limited to addressing the non-compliance. As such, the party making this resubmission: (1) requests that the Office apply the previously-paid fee set forth in 37 CFR 1.290(f), or (2) states that no fee is required to accompany this resubmission as the undersigned is again making the fee exemption statement set forth in 37 CFR 1.290(g).

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Mylan v. Regeneron, IPR2022-01226
Page 151

Samsung Bioepis Exhibit 1017, pg. 151

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	16159282

Signature	/Michael Berry/		
Name/Print	Michael Berry	Registration Number (if applicable)	

Examiner Signature		Date Considered	

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Electronic Acknowledgement Receipt	
EFS ID:	36133892
Application Number:	16159282
International Application Number:	
Confirmation Number:	8618
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First Named Inventor/Applicant Name:	
Correspondence Address:	- - - - - - - - - - -
Filer:	Michael Berry
Filer Authorized By:	
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Receipt Date:	28-MAY-2019
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Time Stamp:	18:48:57
Application Type:	

Payment information:

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Expert_Opinion_2009_Dixon.PDF	309524 2c3c83ba00e8ab387c5811f4e8a8cd525b78e5c8	no	8
Warnings:					
Information:					
2	Non Patent Literature	REGN_News_2009_9_14_General_Releases.PDF	19451 e3bd0705cfeb86d02dd060253ce18d308faa9985	no	2
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Information:					
3	Concise Description of Relevance	Concise_Description_of_Relevance_Dixon.pdf	126013 799231529e1c7dc8e3ca6157997f40484631c099	no	2
Warnings:					
Information:					
4	Concise Description of Relevance	Concise_Description_of_Relevance_Regeneron.pdf	129770 e0e1d202349cb183cbcc9d2d4a02388680c27991	no	3
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Information:					
5	Concise Description of Relevance	Concise-description-generated.pdf	29880 ae58db77ab1df26a286651d7edc3d47bd40693a5	no	3
Warnings:					
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6	Third-Party Submission Under 37 CFR 1.290	Third-party-preissuance-submission.pdf	42846 a9a51a283af49cfe20e69f4f802c10f06a583271	no	3
Warnings:					
Information:					
7	Request for Notification of Non-compliant Third-Party Submission	Third-party-notification-request.pdf	19739 7952fd2c976baa92be77b598e0026bbcd475d8a4	no	1
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Total Files Size (in bytes):	677223

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Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT01012973

Recruitment Status : Completed
First Posted : November 13, 2009
Results First Posted : November 22, 2012
Last Update Posted : November 3, 2014

Sponsor:
Bayer

Collaborator:
Regeneron Pharmaceuticals

Information provided by (Responsible Party):
Bayer

Study Details | Tabular View | Study Results | Disclaimer
How to Read a Study Record

Mylan Exhibit 1017
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Page 156

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Study Description

Go to

Brief Summary:

To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion

Condition or disease	Intervention/treatment	Phase
Retinal Vein Occlusion	Biological: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Other: Sham treatment	Phase 3

Study Design

Go to

Study Type :	Interventional (Clinical Trial)
Actual Enrollment :	177 participants
Allocation:	Randomized
Intervention Model:	Parallel Assignment
Masking:	Triple (Participant, Investigator, Outcomes Assessor)
Primary Purpose:	Treatment
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Study Start Date :	October 2009
Actual Primary Completion Date :	February 2011
Actual Study Completion Date :	February 2012

Resource links provided by the National Library of Medicine

NLM

MedlinePlus related topics: Edema

Drug Information available for: Aflibercept Ziv-aflibercept

U.S. FDA Resources

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Arms and Interventions

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Arm	Intervention/treatment
Experimental: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.	Biological: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Intravitreal injection. Weeks 0 to 20 of Aflibercept Injection every 4 weeks; Weeks 24 to 52 every 4 weeks PRN (pro re nata, on demand); plus additional on Week 60 and 68. Other: Sham treatment Sham treatment. Weeks 0 to 52 sham treatment every 4 weeks; plus additional on Week 60 and 68.
Sham Comparator: Sham treatment Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.	Other: Sham treatment Sham treatment. Weeks 0 to 52 sham treatment every 4 weeks; plus additional on Week 60 and 68.

Outcome Measures

Go to

Primary Outcome Measures :

1. Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures [Time Frame: Baseline and Week 24]

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Defined study baseline range of Early Treatment Diabetic Retinopathy Study (ETDRS) Best Corrected Visual Acuity (BCVA) letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. Nominator = (Number of participants who maintained vision * 100); Denominator = Number of participants analyzed.

Secondary Outcome Measures :

1. Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF) [Time Frame: Baseline and Week 24]

   Defined study baseline range of ETDRS Best Corrected Visual Acuity letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. However, because this was assessed at the screening visit, subjects may have had a higher BCVA recorded at the baseline visit and would not have been excluded from the study.

2. Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF [Time Frame: Baseline and Week 24]
3. Percentage of Participants Who Developed Neovascularization During the First 24 Weeks [Time Frame: From baseline until Week 24]

   Formation of blood vessels in the anterior segment, optic disc, or elsewhere in the fundus up to Week 24

4. Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF [Time Frame: Baseline and Week 24]

   The NEI VFQ-25 total score ranges from 0-100 with a score of 0 being the worst outcome and 100 being the best outcome. The NEI VFQ questionnaire is organized as a collection of subscales which are all scored from 0-100. To reach the overall composite score, each sub-scale score is averaged in order to give each sub-scale equal weight

5. Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF [Time Frame: Baseline and Week 24]

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EQ-5D is a quality of life questionnaire based on a scale from -0.594 (worst) to 1.00 (best).

Eligibility Criteria

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Ages Eligible for Study:	18 Years and older (Adult, Older Adult)
Sexes Eligible for Study:	All
Accepts Healthy Volunteers:	No

Criteria

Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness ≥ 250 µm on optical coherence tomography (OCT)
- Adults ≥ 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis

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- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

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Publications of Results:

Holz FG, Roider J, Ogura Y, Korobelnik JF, Simader C, Groetzbach G, Vitti R, Berliner AJ, Hiemeyer F, Beckmann K, Zeitz O, Sandbrink R. VEGF Trap-Eye for macular oedema

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secondary to central retinal vein occlusion: 6-month results of the phase III GALILEO study. Br J Ophthalmol. 2013 Mar;97(3):278-84. doi: 10.1136/bjophthalmol-2012-301504. Epub 2013 Jan 7. Erratum in: Br J Ophthalmol. 2015 Dec;99(12):1746.

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Responsible Party:	Bayer
ClinicalTrials.gov Identifier:	NCT01012973 History of Changes
Other Study ID Numbers:	14130
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Keywords provided by Bayer:
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- best-corrected visual acuity

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Retinal Vein Occlusion	Thrombosis
Retinal Diseases	Embolism and Thrombosis
Eye Diseases	Vascular Diseases
Venous Thrombosis	Cardiovascular Diseases

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Intravitreal bevacizumab (Avastin) in the treatment of macular edema secondary to retinal vein occlusion

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Objective: To evaluate efficacy and safety of intravitreal injections of bevacizumab in the treatment of macular edema secondary to retinal vein occlusion (RVO).

Methods: Prospective study, noncomparative, interventional case series. Twelve consecutive patients (12 eyes) with macular edema associated with nonischemic retinal vein occlusion were treated with intravitreal bevacizumab (1.25 mg). All subjects underwent standardized ophthalmic evaluation at baseline and at weeks 1, 4, 12, and 24, consisting of visual acuity (VA) measurement using ETDRS charts, and imaging with ocular coherence tomography evaluating changes in foveal thickness (FT) and macular volume (MV).

Results: The median age was 66 years (± 4.16), and the median duration of symptoms was 4 months (± 1.81). There were six cases of inferior branch vein occlusion and six cases of superior branch retinal vein occlusion. Mean VA improved from 1.32 ± 0.24 (logMAR values) at baseline to 0.8 ± 0.15 ($p = 0.0003$) at the 6-month follow-up. The macular edema responded promptly, and a trend to restoration of normal macular anatomy was observed at by the seventh day. Mean FT improved from 615.50 ± 116.29 microns to 420 ± 72.53 microns ($p = 0.001$), and the mean MV improved from 19.81 ± 2.31mm3 to 9.23 ± 1.38 ($p = 0.0001$) at the 6-month follow-up.

Keywords: Bevacizumab, retinal vein occlusion, intravitreal injection, vascular endothelial growth factor

Introduction

Retinal vein occlusion (RVO) is the second most common retinal vascular disease, after diabetic retinopathy. Visual loss may result from ischemic damage and/or macular edema. Early treatment may be required to improve vision because longstanding macular edema results in irreversible photoreceptor damage (CRVO 1995). Intravitreal triamcinolone is a treatment option that has demonstrated promising short-term results for the management of macular edema associated with RVO (Mohammed et al 2007). A multicenter, randomized and controlled clinical trial (Standard Care Versus Corticosteroid for Retina Vein Occlusion Study) is currently underway.

Retinal vein occlusion is associated with varying amounts of retinal ischaemia and, consequently, increased concentrations of vascular endothelial growth factor (VEGF) (Hayreh 1983). Early case reports on bevacizumab showed an increase in visual acuity (VA) and a decrease in macular edema secondary to exudative age-related macular degeneration (AMD) and central retinal vein occlusion (CRVO) (Rosenfeld et al 2005a). A nonrandomized study of intravitreal bevacizumab in patients with CRVO resulted in reduced macular swelling and increased VA (Iturralde et al 2006). However, because a physiological level of vascular endothelial growth factor may be necessary to maintain the homeostasis of the retina, care might be required to avoid the possible negative consequences of a complete blockade of VEGF.

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These reports, along with results from preclinical and human studies that suggest a possible role of VEGF in RVO and the absence of a proven therapy, prompted us to investigate the effects of intravitreal bevacizumab injection in patients with macular edema associated with RVO.

Methods

Study design

This was a prospective, consecutive, noncomparative study that adhered to the Declaration of Helsinki and which was approved by our institutional review board. An intravitreal off-label bevacizumab injection was recommended. The Spanish Ministry of Health and Consumer Affairs approved compassionate use. Patients were fully informed verbally about the experimental nature of the treatment and they signed an informed consent form.

Cases were recruited from the Hospital Universitari de Bellvitge (Barcelona, Spain) from January–March 2007. Inclusion criteria were: 1) patients aged 50 or older 2) macular edema secondary to nonischemic RVO and 3) VA between 20/400 and 20/50 (Snellen equivalent). Exclusion criteria were: 1) history of retinal surgery or photocoagulation; 2) any history of a thromboembolic event; 3) bleeding disorders; and 4) use of anticoagulative medication other than aspirin. No patient refused treatment.

A comprehensive ophthalmic evaluation was performed; it included a medical history review, best corrected visual acuity testing (using ETDRS charts), slit-lamp biomicroscopy, dilated funduscopic examination using a 78-diopters lens and time domain ocular computed tomography (OCT) (Carl Zeiss Meditec, Dublin, CA, USA) that consisted of an acquisition protocol "Radial lines" (6 linear, 6 mm scans oriented at intervals of 30° and centered on the foveal region). Macular maps were obtained using the "retinal thickness/volume" analysis protocol, and values for central foveal thickness (FT) and total macular volume (MV) were recorded. Follow-up examinations were scheduled at weeks 1, 4, 12, and 24 post-injection, or on demand, if a decrease in VA was noted by the patient. These follow-up examinations used exactly the same procedures as those used in the baseline visit. The incidence of adverse events were monitored throughout the study. The effects of treatment, both on VA and on anatomical changes in the macula shown by OCT, were evaluated. There was no loss of follow-up.

Treatment procedure

Patients received an intravitreal dosage of bevacizumab of 1.25 mg (0.05 mL) at baseline and once every four weeks if OCT indicated macular swelling (quantitatively characterized by a macular thickness larger than 250 microns in any of the six radial scans). All treatments were performed in the office using topical anaesthesia (tetracaine+oxibuprocaine) under sterile conditions. Bevacizumab was injected (using a 30-G needle) through the inferotemporal pars plana, 3.5 mm (pseudophakic) or 4 mm (phakic) posterior to the limbus. A drop of ofloxacine was applied to the affected eye immediately after the procedure and again every 6 hours for 4 days.

Statistical data analysis

All data were collected in an Microsoft Excel 2000 spreadsheet (Microsoft Corporation, Spain). For statistical analysis, the Wilcoxon test was performed. VA measurements were converted to logMAR equivalents to perform analysis. $p < 0.05$ was considered significant.

Results

Twelve patients (seven women and five men) were included. The median age was 66 years and the median duration of symptoms prior to treatment was 4 months. Vein occlusion was located at the inferior branch in six patients and at the superior branch in the remaining six patients. There was a history of hypertension in four patients. All patients completed the 24-week follow-up examination; their baseline characteristics are summarized in Table 1.

Best corrected visual acuity (BCVA) data obtained over the course of the study are summarized in Table 2. Evaluation of BCVA revealed significant improvement at all times compared with baseline. Mean VA improved from 1.32 ± 0.24 (logMAR values) at baseline, to 0.8 ± 0.15 ($p = 0.0003$) at the 6-month follow-up. The macular edema responded promptly, and a reduction in the submacular fluid was observed at the seventh day. At baseline, the mean FT was 615.50 ± 116.29 microns; it declined to 420 ± 72.53 microns ($p = 0.001$). The mean MV improved from 19.81 ± 2.31 mm^3 to 9.23 ± 1.38 mm^3 ($p = 0.0001$) at the 6-month follow-up. Mean changes in parameters recorded by OCT on weeks 1, 4, 12, and 24 post-injection are summarized in Table 3. Overall, four patients were retreated: 2 patients received two consecutive injections of intravitreal bevacizumab, and two patients received three injections. No ocular or systemic adverse events were observed.

Discussion

This study demonstrates the early and clinically relevant benefits of bevacizumab injection for macular edema due to RVO. In this prospective case series, we found that intravitreal

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Table 1 Baseline characteristics

Case, age, sex	Affected ye, localization	Duration (months)	logMAR BCVA	OCT FT (microns)	OCT MV (mm^3)
1, 64, F	OD, inferior	2.5	0.63	664	12.91
2, 63, F	OD, inferior	5	1.7	545	22.17
3, 75, M	OD, superior	6	1.05	410	16.65
4, 57, M	OD, superior	3	1.63	667	21.23
5, 67, F	OD, superior	2.5	1.96	601	20.82
6, 66, F	OS, inferior	2	0.62	763	11.9
7, 61 F	OD, superior	3	1.6	495	21.16
8, 75, F	OD, superior	4	1.04	630	15.64
9, 77, M	OS, superior	4	1.62	239	20.22
10, 66, M	OS, inferior	7	1.34	885	12.97
11, 59, M	OS, inferior	6	1.3	498	11.2
12, 79, F	OS, inferior	7	0.96	998	19.81
Median: 66 SD: 7,26 Confidence intervals (CI): 4.16		Median: 4 SD: 1.81 CI: 1.02	Median: 1.32 SD: 0.43 CI: 0.24	Median: 615.50 SD: 205.547 CI: 116.29	Median: 19.81 SD: 4.10 CI: 2.31

Abbreviations: F, female; M, male; SD, standard deviation; OD, right eye; OS, left eye; BCVA, best corrected visual activity; FT, foveal thickness; MV, macular volume.

injections of bevacizumab led both to a significant reduction of FT, as well as to an improvement of visual acuity in patients with RVO. A beneficial effect of intravitreal bevacizumab was observerd as early as the first week and over a 6-month follow-up period.

Our study supports the preliminary results of several recently published papers. The most detailed data on the natural history of CRVO were provided by the Central Vein Occlusion Study Group (CRVO 1995). Clinical outcomes of every new treatment option for CRVO must match with these data. In the natural course of CRVO, only 19% of patients with initial visual acuity of less than 20/200 had a chance of visual acuity of better than 20/200. Patients presenting with initial visual acuity between 20/200 and 20/50 had improvement to better than 20/50 in 19% of cases; in 44% of cases acuity stayed between 20/200 and 20/50. The visual acuity of only 37% of patients became worse than 20/200. Compared with these data, patients treated with intravitreal injections of bevacizumab showed much greater improvement. Priglinger and colleagues (2007) reported improvement in visual acuity from 20/250 at baseline to 20/80 at the 6-month follow-up ($p < 0.001$) in a group of 46 CRVO patients. Mean central retinal thickness decreased from 535 ± 48 microns at baseline to 323 ± 116 microns at the 6-month follow-up (Priglinger et al 2007). In a series of 30 CRVO patients followed for 6 months, Jason and colleagues (2007) reported improvement in VA from 20/394 at baseline to 20/313 at the 3-month follow-up, ($p < 0.05$) and no significant changes after the fourth month. This indicates that bevacizumab represents an effective treatment option for CRVO and that the drug may improve the long-term prognosis of CRVO.

The intravitreal use of bevacizumab may provide anatomical and functional amelioration of the macula in patients with macular edema due to RVO. The electrical responses in the fovea and parafovea of the multifocal electroretinography recording depict a significant improvement at 1 and 3 months after the injection (Moschos and Moschos 2008).

Table 2 logMAR BCVA (baseline and 1, 4 12, and 24 weeks post-injection)

logMAR BCVA baseline	logMAR BCVA, week 1	logMAR BCVA week 4	logMAR BCVA week 12	logMAR BCVA week 24
0.63	0.35	0.17	0.23	0.43
1.7	1.3	0.75	0.77	0.77
1.05	1.01	1.03	1	0.89
1.63	1.47	1.2	1.35	1.17
1.96	0.4	0.43	0.61	0.53
0.62	0.36	0.17	0.23	0.42
1.6	1.2	0.75	0.78	0.76
1.04	1.02	1.02	1	0.86
1.62	1.48	1.3	1.36	1.19
1.34	1	0.78	0.86	0.83
1.3	1.28	1.24	1.28	1.25
0.96	0.5	0.42	0.62	0.56

Note: p (baseline-week 24) = 0.0003
Abbreviation: BCVA, best corrected visual acuity.

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Table 3 FT (microns) and MV (mm3) (baseline and 1, 4, 12, and 24 week post-injection)

Case 1	Baseline	Week 1	Week 4	Week 12	Week 24
FT	664	220	166	167	189
MV	12.91	8.48	7.87	8.38	8.34
Case 2					
FT	545	440	165	481	654
MV	22.17	6.96	6.64	9.33	8.35
Case 3					
FT	410	166	496	509	578
MV	16.65	12.17	13.2	13.95	12.95
Case 4					
FT	667	565	459	500	475
MV	21.23	17.32	15.16	15.69	14.63
Case 5					
FT	601	436	175	501	420
MV	20.82	6.66	6.83	9.2	9.5
Case 6					
FT	763	400	170	456	432
MV	11.9	7.47	6.86	7.37	10.56
Case 7					
FT	495	175	195	225	235
MV	21.16	5.95	5.63	8.32	7.35
Case 8					
FT	630	220	460	335	342
MV	15.64	11.16	12.1	12.94	10.55
Case 9					
FT	239	442	336	402	389
MV	20.22	16.31	12.1	14.68	12.48
Case 10					
FT	885	296	450	425	420
MV	12.97	6.25	10.91	10.55	8.95
Case 11					
FT	498	345	450	356	352
MV	11.2	10.72	5.82	10.97	7.55
Case 12					
FT	998	425	182	522	425
MV	19.81	5.55	5.85	8.1	6.98

Notes: p (baseline- week 24): FT = 0.001 MV = 0.0001
Abbreviations: FT, foveal thickness; MV, and macular volume.

Intravitreal injection of triamcinolone acetonide (TA) is another treatment option aimed to reduce macular edema after RVO. Several recent studies report favorable effects of intravitreal injection (4–20 mg) of TA on the course of RVO (Gregory et al 2006). The extent and duration of the effect of intravitreal injection of TA depends on the dose used and the presence of retinal ischemia (Jonas et al 2005). Repeated intravitreal injections of TA are possible; however, after repeated treatments, the effect on reduction of retinal thickness and increase in visual acuity are reduced (Boyd et al 2002; Kupperman et al 2007). Furthermore, although apparently improving the clinical outcome of RVO, repeated intravitreal injections of TA are associated with many potential complications, such as elevated intraocular pressure and cataract formation, which may ultimately decrease the long-term prognosis of RVO (Goff et al 2006). In contrast to intravitreal injection of TA, several injections of bevacizumab appear to have no drug-related complications. However, complications related to repeated intravitreal injections (eg, endophthalmitis, retinal tear, and lens trauma) must be taken into account (Jaissle et al 2006). Fortunately, none of these complications occurred in the present case series; this may be due to the thorough prophylactic, antiseptic regimen applied in our institution to minimize the likelihood of bacterial contamination.

The use of anti-VEGF agents in retinal disease has become increasingly common since the approval (in 2004 and 2006, respectively) of pegaptanib and ranibizumab for age-related maculopathy. These agents are currently being studied for their efficacy against macular edema due to RVO. The anti-VEGF agent most studied in regard to RVO is bevacizumab. Off-label intravitreal injection of bevacizumab was first reported in 2005 to represent a potential therapy for macular edema secondary to CRVO (Rosenfeld et al 2005b). Since then, several additional publications have reported favorable short-term results for reduction of macular edema and improvement of vision in patients with RVO (Spandau et al 2006; Pau et al 2007).

Our results suggest a possible short-term benefit for macular architecture and VA; however, it is also clear that such benefits are transient. Continuous VEGF suppression may be required to sustain beneficial effects observed in the short term and the risks associated with multiple intravitreal injections need to be considered. Our data suggest that patients may require several injections to maintain efficacy. Three to four months after the most recent injection, worsening VA was detected in about half of the cases. The data in this study suggest that a single injection of intravitreal bevacizumab has a limited beneficial effect for approximately two months in most patients.

Although VEGF and its receptors represent potential targets for pharmacologic intervention, several important questions remain. Does VEGF play a role in the formation of vascular shunts across ischemic areas? Does continuous blockage of VEGF have a negative effect over the long term? Furthermore, recurrent macular edema may occur in patients

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with RVO following treatment with bevacizumab; in some cases, the recurrent macular edema may be more severe than the pre-treatment macular edema (a phenomenon known as "rebound" macular edema) (Matsumoto et al 2007).

The changes observed throughout the present study may provide important clues about drug effects and duration. Favorable macular changes, documented by OCT, were evident as soon as day 7 post-injection. While these improvements were maintained at post-injection week 4, there was a clear tendency for macular edema to recur around week 12; this suggests, therefore, that reinjections might be considered at some point during this period when a 1.25 mg dose regimen is used in setting of ischemic or nonischemic RVO.

The present study does have some limitations that must be recognized: there was no control group; we included only 12 patients and there was only a limited follow-up. However, the promising results reported here indicate that further studies of intravitreal bevacizumab injection for the management of ischemic or nonischemic RVO are justified. Future well-designed studies will help to establish the role of antiangiogenic therapy in the management of RVO.

Disclosure

The authors report no conflicts of interest in this work.

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Vascular endothelial growth factor Trap-Eye for diabetic macular oedema

Peter K Kaiser

Diabetic macular oedema (DMO) is the leading cause of visual loss in working-aged Americans, and as such successful treatment is of major public health importance. The gold standard is laser photocoagulation based on the landmark Early Treatment Diabetic Retinopathy Study (ETDRS).[1] In the ETDRS, laser treatment reduced the 3-year risk of moderate visual loss by 50%; however, the standard teaching is that patients in the ETDRS rarely improved vision. In fact, in the published ETDRS manuscripts to date, only around 10% of patients gained more than three lines of vision. This led to the prevailing thinking that laser prevents vision loss, but rarely results in visual improvement. Thus, newer therapies have been explored in an effort to improve outcomes in this common and devastating condition.

Vascular endothelial growth factor (VEGF) was one of the first cytokines implicated in diabetic retinopathy and DMO with elevated VEGF levels found in patients with active disease.[2] Increased retinal capillary permeability due to breakdown of the blood–retina barrier have been shown to be mediated by VEGF.[3] Thus, VEGF appears to be a good target to prevent DMO and progression of diabetic retinopathy.

Several anti-VEGF agents have been evaluated in off-label case series and small-scale clinical trials to treat DMO. In a randomised, phase 2 clinical trial of 172 patients, pegaptanib (Macugen, Eyetech, New York), a VEGF165 specific blocker, resulted in a mean improvement in vision of +4.7 letters with 34% gaining 10 letters of more at 36 weeks.[4] Similarly, the randomised, phase 2 DRCR.net trial of 121 patients with bevacizumab (Avastin, Genentech, South San Francisco, California), a pan-VEGF antibody, reported a mean improvement in vision of +5 letters with 33% having greater than 10 letters of improvement at 12 weeks.[5] Finally, the phase 1/2 Ranibizumab for (O)edema of the Macula in Diabetes (READ) study evaluated ranibizumab (Lucentis, Genentech), an antibody fragment that blocks all forms of VEGF, and reported +12.3 letters of visual improvement at month 7. A pivotal Phase 3 trial testing ranibizumab for DMO is currently under way. In this issue ***(see page 144)***, another VEGF blocker has shown early success in the management of DMO—VEGF Trap-Eye.[6]

Traps consist of two extracellular cytokine receptor domains fused together to a human immunoglobulin G (IgG).[7] VEGF Trap-Eye is a soluble fusion protein that combines ligand-binding elements taken from the extracellular components of VEGF receptors 1 and 2 fused to the Fc portion of IgG1.[8] This protein contains all human amino acid sequences, which minimises the potential for immunogenicity in human patients. Similar to bevacizumab and ranibizumab, VEGF Trap-Eye blocks all isoforms of VEGF and placental growth factor. In the small phase 1, open-label study by Do and colleagues, five patients with DMO were treated with VEGF-Trap Eye with good short-term results and safety.

One may ask why one should develop another extracellular VEGF blocker when we already have bevacizumab and ranibizumab. VEGF Trap-Eye has several features that make it appealing in comparison with the currently available therapeutics. The key feature is the binding constant for VEGF is approximately 0.5 pM Kd, considerably higher than ranibizumab, bevacizumab or even native VEGF receptors. This higher binding affinity translates into greater activity at lower biological levels, and consequently a longer duration of action. Some studies have suggested as long as 10 weeks.[9] This longer duration is very important, as diabetic retinopathy is a chronic condition, and most patients are working. All the anti-VEGF DMO studies to date indicate that when the therapy was stopped, vision returned to baseline, and any gains in retinal thickness and fluorescein leakage were reversed. Thus, continuous treatment may be necessary to maintain the improvements in outcomes. These repeated intravitreal anti-VEGF injections will put a strain on both the healthcare system and patients.

With all anti-VEGF agents, systemic safety needs to be evaluated. All known anti-VEGF therapeutics are detectable in the systemic circulation, often with levels high enough to block all native VEGF. Concerns have been raised about systemic effects including stroke, myocardial infarction and hypertension. Current anti-VEGF studies have been too small to truly evaluate the safety of these drugs in the general population. While a recent meta-analysis with all the randomised ranibizumab studies failed to reveal any increased risk of these events, it also does not mean there is not an increased risk, and we all need to be cognisant of this fact (D Boyer, American Academy of Ophthalmology Meeting, Atlanta, Georgia, 2008).

Finally, one important fact overlooked by many in the ETDRS results and more importantly in the statistical planning for many current clinical trials for DMO is that 85% of the patients in the ETDRS could not improve three lines, since they had vision better than 20/40 at the start of the study.[1] In fact, in a subset of 105 ETDRS patients with definite centre thickening on fundus photographs, less severe retinopathy and a visual acuity letter score <20/32 at baseline, the median change in the visual acuity letter score was +4 letters, with 30% improving 10 or more letters at 2 years.[10] Similarly, the DRCR.net reported at 2 years a mean improvement of 1 letter with 18% gaining three or more lines.[10] So, our old standby, laser, really works better than we thought! All studies with anti-VEGF to date have been too short to truly evaluate the long-term efficacy of this treatment. The DRCR.net showed us that a treatment we thought was better than laser was only better in the short term, and long-term follow-up was necessary to actually show the benefit of laser over steroids in this particular patient population. Thus, we need to wait for the results of ongoing studies with longer follow-up before we declare anti-VEGF therapy a new paradigm in DMO management.

The addition of VEGF-Trap Eye as a possible treatment for DMO is exciting, as it has some appealing features over current anti-VEGF therapies. However, our enthusiasm has to be tempered by

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the short-term nature of this study, the theoretical risks of anti-VEGF therapy and the possible need for continuous therapy to achieve visual gains. Nevertheless, there are many patients who do not respond to laser, and continued exploration into new treatments and even treatment combinations is warranted.

Funding: None.

Competing interests: PKK's employer, the Cole Eye Institute, has received research grant support from Genentech on his behalf. PKK serves on the Scientific Advisory Boards of Genentech and Regeneron. His participation in these boards has been approved and disclosed to the Cleveland Clinic's Conflict of Interest Committee.

Br J Ophthalmol 2009;**93**:135–136. doi:10.1136/bjo.2008.144071

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Cover illustration

Lord Shiva's third eye

The cover illustration depicts the Lord Shiva, the third god of the Hindu Trinity (Trimurti), represented by Lord Brahma, Lord Vishnu and Lord Shiva, the Creator, the Preserver and the Destroyer of the universe, respectively. Some contend that the word "destroyer" is used in error as dissolution for creation, as autumn is to spring, is an essential part of the cyclic process of creation, preservation, dissolution and re-creation.

The image of Shiva, as portrayed among Hindus, contains common symbols representative of his superiority. One of these symbols is his third eye, seen in the centre of his forehead; hence he is often referred to as Tryambaka Deva (literally meaning "three-eyed lord"). His right eye is believed to be the sun, the left eye is the moon and his third eye represents fire. His left and right eyes indicate his activity in the physical world. Often Shiva is shown with half open eyes, signifying the never ending, ongoing nature of the birth and destruction of the universe. When the eyes are completely closed it signifies the dissolution of the universe and when it is completely open a new cycle of creation begins.

The third eye can detect evil, even when it is not immediately apparent and the fire that emanates from it can annihilate evil. It also symbolises his activity in the metaphysical, spiritual world and represents the power of knowledge. This eye is seen to be the source of his untamed energy. Once while Shiva was in the midst of worship, the love god, Kama, distracted him. In his anger he opened his third eye, and fire from the eye devoured Kama, until Parvati (Shiva's wife, also known as Kali mata) saved him. For these reasons Shiva is seen as the "destroyer". The power of this eye is so great that it is feared by wrongdoers.

It is said to have appeared when Parvati playfully blindfolded him with her two hands, while he was deep in meditation. Immediately the universe plunged into darkness and chaos reigned supreme. To prevent impending catastrophe, Shiva formed a third eye from which fire emerged to recreate light and order, hence saving the world from inevitable disaster. Throughout imagery of Shiva this eye is depicted as closed or by three horizontal lines in the middle of his forehead. Many Hindus wear a tilak between the eyebrows to represent the third eye, as it is seen as a sign of enlightenment.

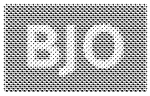
Lord Shiva is always shown with a snake (Vasuki Naga, symbol of yogic powers) coiled three times (for the past, present and future) around his neck.

Neeru Dhillon, Arun D Singh, Harminder S Dua

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Vascular endothelial growth factor Trap-Eye for diabetic macular oedema

Peter K Kaiser

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Title of Invention:	USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS
First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
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1	Transmittal Letter	0725US6_2020-01-27_Supp_IDS_trans_REGN-008CIPCON5.pdf	51139 825ff5729d58ffff621e277e66a672d99bd5932f	no	2
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INFORMATION DISCLOSURE STATEMENT	Attorney Docket No.	REGN-008CIPCON5
	Confirmation No.	8135
	First Named Inventor	George D. Yancopoulos
	Application Number	16/397,267
	Filing Date	April 29, 2019
	Group Art Unit	
	Examiner Name	
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:

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. All documents with the exception of documents (1), (2), (9), (11) and (12) in the non-patent literature were previously submitted and copies are not enclosed. These documents are being relisted on the PTO/SB/08A form to complete the NPL cite from the originally submitted version, for example, article submitted while "In Press".

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

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☒ 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-008CIPCON5.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 27 January 2020

By: /Karl Bozicevic, Reg. No. 28,807/
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	George D. Yancopoulos
				Art Unit	
				Examiner Name	
Sheet	1	of	4	Attorney Docket Number	REGN-008CIPCON5

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				Examiner Name	
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Sheet	4	of	4	Attorney Docket Number	REGN-008CIPCON5

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
	23	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 12 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_12212010_27443.1)	

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.

U.S. National Library of Medicine
ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
- Additions will be displayed in *green*.
- The yellow choices in the table indicate the study versions currently compared below. A yellow row indicates the study version being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: January 18, 2013 (v21)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	2009-010973-19 [EudraCT Number]

Study Status

Record Verification:	January 2013
Overall Status:	Completed
Study Start:	October 2009
Primary Completion:	February 2011 [Actual]
Study Completion:	February 2012 [Actual]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Results First Submitted:	October 23, 2012
	October 23, 2012

Results First Submitted that Met QC Criteria:	
Results First Posted:	November 22, 2012 [Estimate]
Certification/Extension First Submitted:	January 26, 2012
Certification/Extension First Submitted that Met QC Criteria:	January 26, 2012
Certification/Extension First Posted:	January 30, 2012 [Estimate]
Last Update Submitted that Met QC Criteria:	January 18, 2013
Last Update Posted:	January 24, 2013 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion

CRVO
VEGF Trap-Eye
best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	177 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.	Biological: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Intravitreal injection. Weeks 0 to 20 of Aflibercept Injection every 4 weeks; Weeks 24 to 52 every 4 weeks PRN (pro re nata, on demand); plus additional on Week 60 and 68. Sham treatment Sham treatment. Weeks 0 to 52 sham treatment every 4 weeks; plus additional on Week 60 and 68.
Sham Comparator: Sham treatment Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the	Sham treatment Sham treatment. Weeks 0 to 52 sham treatment every 4 weeks; plus additional on Week 60 and 68.

Arms	Assigned Interventions
study retreatment criteria at Week 60 and 68.	

Outcome Measures

[See Results Section]

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness ≥ 250 µm on optical coherence tomography (OCT)
- Adults ≥ 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis

- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Marburg, Hessen, Germany, 35037

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66421

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Hungary

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Budapest, Hungary, 1133

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

Japan, Aichi

Nagoya, Aichi, Japan, 466-8560

Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

Suita, Osaka, Japan, 565-0871

Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

IPDSharing

Plan to Share IPD:

References

Citations:

Links:

Available IPD/Information:

Study Results

Participant Flow

Reporting Groups

	Description
Aflibercept Injection First, Then Aflibercept Injection	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment First, Then Aflibercept Injection	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Overall Study

	Aflibercept Injection First, Then Aflibercept Injection	Sham Treatment First, Then Aflibercept Injection
Started	106	71
Participants Received Treatment	104 [1]	68 [1]
Fulfilled Requirements of FAS Population	103 [2]	68 [2]
Completed Week 24, From FAS	97	57
Completed Week 52, From FAS	91	52
Completed	90	52
Not Completed	16	19
Adverse Event	5	5
Lack of Efficacy	0	5
Lost to Follow-up	1	0

	Aflibercept Injection First, Then Aflibercept Injection	Sham Treatment First, Then Aflibercept Injection
(Overseas travel - indefinite period)		
Increase in vis. acuity, never injected	0	1
Protocol Violation	5	2
Withdrawal by Subject	4	6

[1] Safety Population: Participants received treatment

[2] Full Analysis Set (FAS) Population: Participants received treatment with post baseline measurements

Baseline Characteristics

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Baseline Measures

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Overall Number of Participants		104	68	172
Age Continuous Mean (Standard Deviation) Unit of measure: Years	Number Analyzed	104 Participants	68 Participants	172 Participants
		60.0 (12.3)	63.8 (13.3)	61.5 (12.8)
Sex: Female, Male Measure type: Count of Participants Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
	Female	45 43.27%	31 45.59%	76 44.19%
	Male	59 56.73%	37 54.41%	96 55.81%
Ethnicity (NIH/OMB) Measure type: Count of Participants Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
	Hispanic or Latino	4 3.85%	1 1.47%	5 2.91%
	Not Hispanic or Latino	100 96.15%	66 97.06%	166 96.51%
	Unknown or Not Reported	0 0%	1 1.47%	1 0.58%
Baseline Best Corrected Visual Acuity (BCVA) letter scores [1] Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		53.5 (15.7)	50.9 (15.4)	52.5 (15.6)
	[1]	Information retrieved from all baseline participants. Only participants with a ETDRS (Early Treatment Diabetic Retinopathy Study) Best Corrected Visual Acuity letter score of 73 to 25 (= Acuity of 20/40 to 20/320) in the study eye at 4 meters were included; a higher score represents better functioning.		
		104 Participants	68 Participants	172 Participants

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Number of participants with baseline retinal perfusion [1] Measure type: Number Unit of measure: Participants	Number Analyzed			
Perfused		90	54	144
Nonperfused		7	7	14
Indeterminate		7	7	14
	[1] Retinal perfusion defined as less than 10 disc areas of capillary nonperfusion using fluorescein angiography (FA)			
Baseline Retinal Thickness by Optical Coherence Tomography (OCT) Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		682.78 (233.36)	638.66 (224.69)	665.34 (230.33)
Baseline intraocular pressure Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		15.2 (2.8)	14.4 (2.7)	14.9 (2.8)
Number of participants with time since Central retinal vein occlusion (CRVO) diagnosis Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
>= 2 months		46	33	79
< 2 months		56	35	91
Missing		2	0	2

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Baseline National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) total score [1]	Number Analyzed	104 Participants	68 Participants	172 Participants
		79.66 (13.06)	78.94 (14.00)	79.38 (13.40)

[1] The NEI VFQ-25 total score ranges from 0-100 with a score of 0 being the worst outcome and 100 being the best outcome. The NEI VFQ questionnaire is organized as a collection of subscales which are all scored from 0-100. To reach the overall composite score, each sub-scale score is averaged in order to give each sub-scale equal weight.

European questionnaire 5 dimensions (EQ-5D) total score [1] Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		0.87 (0.15)	0.86 (0.16)	0.87 (0.15)

[1] The EQ-5D total score ranges from -0.594 to 1.000 with -0.594 being the worst.

Race Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
Asian		26	15	41
White		75	49	124
Unknown or Not Reported		3	4	7

Outcome Measures

1. Primary Outcome Measure:

Measure Title	

	Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures
Measure Description	Defined study baseline range of Early Treatment Diabetic Retinopathy Study (ETDRS) Best Corrected Visual Acuity (BCVA) letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. Nominator = (Number of participants who maintained vision * 100); Denominator = Number of participants analyzed.
Time Frame	Baseline and Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures Measure Type: Number Unit of Measure: Percentage of participants	60.2	22.1

Statistical Analysis 1 for Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis of difference of Eylea minus Sham of 0 was tested. In the database close after Week 24, basis for primary efficacy evaluation, 56 Sham / 96 Eylea subjects were considered as week 24 completers.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	[Not specified]
	Method	Cochran-Mantel-Haenszel
	Comments	[Not specified]

	Estimation Parameter	CMH adjusted difference

Method of Estimation	Estimated Value	38.3
	Confidence Interval	(2-sided) 95% 24.4 to 52.1
	Estimation Comments	The estimate is calculated as Eylea minus Sham. A positive value shows Eylea showed a higher BCVA total score compared to Sham.

2. Secondary Outcome Measure:

Measure Title	Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF)
Measure Description	Defined study baseline range of ETDRS Best Corrected Visual Acuity letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. However, because this was assessed at the screening visit, subjects may have had a higher BCVA recorded at the baseline visit and would not have been excluded from the study.
Time Frame	Baseline and Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI

	depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF) Measure Type: Mean (Standard Deviation) Unit of Measure: Letters correctly read	71.6 (17.1)	54.3 (20.2)

Statistical Analysis 1 for Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF)

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis was equality in change from baseline to Week 24 in BCVA total letter score between Eylea and Sham. If primary efficacy was successful, secondary efficacy endpoints were tested in a pre-specified fixed sequence testing procedure. Change in BCVA letter

		score was to be tested first in this sequence.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	As primary efficacy evaluation was significant, and this p-value was below significance level of two-sided <.05, the fixed sequence testing did continue with next secondary endpoint.
	Method	ANOVA
	Comments	ANOVA, adjusting for region and baseline BCVA category as fixed factors.

Method of Estimation	Estimation Parameter	Difference in Least square means
	Estimated Value	14.7
	Confidence Interval	(2-sided) 95% 10.8 to 18.7
	Estimation Comments	The difference is calculated as Eylea minus Sham. A positive value indicates Eylea showed a higher change in BCVA total score until week 24 compared to Sham.

3. Secondary Outcome Measure:

Measure Title	Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF
Measure Description	
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	67
Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: microns	-448.58 (256.02)	-169.27 (224.72)

Statistical Analysis 1 for Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF

	Comparison Groups	

Statistical Analysis Overview		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis was equality in change from baseline to Week 24 in central retinal thickness between Eylea and Sham. If primary efficacy was successful, secondary efficacy end points were to be tested in a pre-specified fixed sequence testing procedure. Change in central retinal thickness was to be tested at second place in this sequence.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	As fixed sequence testing did reject nullhypothesis of change from baseline in BCVA until week 24, and this p-value was below significance level of two-sided <.05, the fixed sequence testing did continue with next secondary endpoint.
	Method	ANCOVA
	Comments	ANCOVA, stratified by region and baseline BCVA category, baseline central retinal thickness added as covariate.

Method of Estimation	Estimation Parameter	Difference in Least square (LS) means
	Estimated Value	-239.42
	Confidence Interval	(2-sided) 95% -286.31 to -192.53

	Estimation Comments	The difference is calculated as Eylea minus Sham. A negative value indicates Eylea showed a higher reduction in change in central retinal thickness until week 24 compared to Sham.

4. Secondary Outcome Measure:

Measure Title	Percentage of Participants Who Developed Neovascularization During the First 24 Weeks
Measure Description	Formation of blood vessels in the anterior segment, optic disc, or elsewhere in the fundus up to Week 24
Time Frame	From baseline until Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Percentage of Participants Who Developed Neovascularization During the First 24 Weeks Measure Type: Number Unit of Measure: Percentage of participants		
Any neovascularization	2.9	4.4
Anterior segment neovascularization	1.9	1.5
Neovascularization of the optic disc (NVD)	0.0	0.0
Neovascularization elsewhere in the fundus (NVE)	1.0	2.9

Statistical Analysis 1 for Percentage of Participants Who Developed Neovascularization During the First 24 Weeks

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Nullhypothesis of no difference in development of neovascularizations between Eylea and Sham group was tested. (Any neovascularization)
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	0.5947
	Comments	As fixed sequence testing did reject nullhypothesis of change from baseline in CRT until week 24, and this p-value was not below significance level of two-sided <.05,

		the fixed sequence testing did end with this evaluation.
	Method	Cochran-Mantel-Haenszel
	Comments	Cochrane-Mantel-Haenszel test, stratified by region and baseline BCVA category.

Method of Estimation	Estimation Parameter	CMH adjusted Difference
	Estimated Value	-1.5
	Confidence Interval	(2-sided) 95% -7.4 to 4.4
	Estimation Comments	[Not specified]

5. Secondary Outcome Measure:

Measure Title	Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF
Measure Description	The NEI VFQ-25 total score ranges from 0-100 with a score of 0 being the worst outcome and 100 being the best outcome. The NEI VFQ questionnaire is organized as a collection of subscales which are all scored from 0-100. To reach the overall composite score, each sub-scale score is averaged in order to give each sub-scale equal weight
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often

	as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	96	65
Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: Scores on a scale	7.46 (9.55)	3.55 (9.74)

Statistical Analysis 1 for Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	[Not specified]
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

	Estimation Parameter	Difference in LS means

Method of Estimation	Estimated Value	4.2
	Confidence Interval	(2-sided) 95% 1.7 to 6.8
	Estimation Comments	As the fixed sequence of secondary endpoints stopped with proportion of neovascularizations developed until week 24, 95% confidence interval is only of descriptive nature.

6. Secondary Outcome Measure:

Measure Title	Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF
Measure Description	EQ-5D is a quality of life questionnaire based on a scale from -0.594 (worst) to 1.00 (best).
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment,

	who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	95	64
Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: Scores on a scale	0.029 (0.139)	-0.002 (0.195)

Statistical Analysis 1 for Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	[Not specified]
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Method of Estimation	Estimation Parameter	Difference in LS Means
	Estimated Value	0.044
	Confidence Interval	(2-sided) 95% -0.002 to 0.09
	Estimation Comments	As the fixed sequence of secondary endpoints stopped with proportion of neovascularizations developed until week 24, 95% confidence interval is only of descriptive nature.

Reported Adverse Events

Time Frame	[Not specified]
Adverse Event Reporting Description	[Not specified]

Reporting Groups

	Description
Aflibercept Injection (Until Week 20)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20. Participants were observed until Week 24. Participants in the safety population were at risk.
Sham Treatment (Until Week 20)	Participants received sham treatment administered every 4 weeks from Day 1 through Week 20. Participants were observed until Week 24. Participants in the safety population were at risk.
Aflibercept Injection (Until Week 48)	Participants who continued the study drug until Week 24 received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Participants were observed from Week 24 until Week 52. Participants in the safety population that completed Week 24 were at risk.
Sham Treatment (Until Week 48)	Participants who continued the study drug until Week 24 received sham treatment administered every 4 weeks from Week 24 to Week 48. Participants were observed from Week 24 until Week 52. Participants in the safety population that completed Week 24 were at risk.
Aflibercept Injection Continued (Until Week 68)	Participants on IAI who continued the study drug until Week 52, received 2 mg dose of IAI depending on the study retreatment criteria at Week 52, 60 and 68. Participants were observed starting from Week 52. Participants in the safety population that completed Week 52 were at risk.

Sham Treatment Then Aflibercept Injection (Until Week 68)	Participants on sham treatment switched to IAI, received a 2 mg dose of IAI at Week 52 and depending on the study retreatment criteria at Week 60 and 68. Participants were observed starting from Week 52. Participants in the safety population that completed Week 52 were at risk.

All-Cause Mortality

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)
Total						

Serious Adverse Events

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)
Total	8/104 (7.69%)	8/68 (11.76%)	14/97 (14.43%)	7/57 (12.28%)	4/91 (4.4%)	3/52 (5.77%)
Cardiac disorders						
Aortic valve incompetence [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Cardiac failure [A*]						

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Coronary artery stenosis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Diastolic dysfunction [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Mitral valve incompetence [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Eye disorders						
Blindness unilateral [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Glaucoma [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Iris neovascularisation [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular fibrosis [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular ischaemia [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular oedema [A*]	0/104 (0%)	2/68 (2.94%)	4/97 (4.12%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Retinal vein occlusion [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Visual acuity reduced [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	0/57 (0%)	2/91 (2.2%)	0/52 (0%)
Vitreous detachment [A*]	1/104	0/68	0/97	0/57	0/91	0/52

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Vitreous haemorrhage [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Gastrointestinal disorders						
Diverticular perforation [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Hepatobiliary disorders						
Hepatic function abnormal [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Infections and infestations						
Furuncle [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Gastroenteritis [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Pneumonia [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Vestibular neuronitis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Injury, poisoning and procedural complications						
Fall [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Femur fracture [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Hand fracture [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Humerus fracture [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Radius fracture [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Spinal compression fracture [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Musculoskeletal and connective tissue disorders						
Intervertebral disc protrusion [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Spinal column stenosis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)						
Breast cancer [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Oropharyngeal cancer stage unspecified [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Nervous system disorders						
Paraesthesia [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Syncope [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	2/57 (3.51%)	0/91 (0%)	0/52 (0%)
Transient ischaemic attack [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Respiratory, thoracic and mediastinal disorders						
Dyspnoea [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Laryngeal granuloma [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Pulmonary hypertension [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Surgical and medical procedures						
Ischaemic heart disease prophylaxis [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vascular disorders						
Circulatory collapse [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)

* Indicates events were collected by non-systematic methods.

A Term from vocabulary, MedDRA (14.1)

Other Adverse Events

Frequency Threshold Above Which Other Adverse Events are Reported: 5%

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)
Total	52/104 (50%)	44/68 (64.71%	66/97 (68.04%	30/57 (52.63%	38/91 (41.76%	19/52 (36.54%
Blood and lymphatic system disorders						
Anaemia [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Eye disorders						
Conjunctival haemorrhage [A+]	10/104 (9.62%)	3/68 (4.41%)	3/97 (3.09%)	0/57 (0%)	9/91 (9.89%)	3/52 (5.77%)
Eye irritation [A+]	3/104 (2.88%)	7/68 (10.29%)	4/97 (4.12%)	1/57 (1.75%)	1/91 (1.1%)	2/52 (3.85%)
Eye pain [A+]	12/104 (11.54%)	3/68 (4.41%)	6/97 (6.19%)	2/57 (3.51%)	1/91 (1.1%)	0/52 (0%)
Foreign body sensation in eyes [A+]	6/104 (5.77%)	5/68 (7.35%)	2/97 (2.06%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Lacrimation increased [A+]	3/104 (2.88%)	4/68 (5.88%)	3/97 (3.09%)	4/57 (7.02%)	1/91 (1.1%)	2/52 (3.85%)
Macular fibrosis [A+]	1/104 (0.96%)	1/68 (1.47%)	5/97 (5.15%)	4/57 (7.02%)	0/91 (0%)	3/52 (5.77%)
Macular ischaemia [A+]	7/104 (6.73%)	5/68 (7.35%)	3/97 (3.09%)	1/57 (1.75%)	0/91 (0%)	1/52 (1.92%)
Macular oedema [A+]	2/104 (1.92%)	9/68 (13.24%)	30/97 (30.93%)	7/57 (12.28%)	17/91 (18.68%)	2/52 (3.85%)
Ocular hyperaemia [A+]	5/104 (4.81%)	4/68 (5.88%)	2/97 (2.06%)	1/57 (1.75%)	4/91 (4.4%)	1/52 (1.92%)
Optic disc vascular disorder [A+]	5/104 (4.81%)	3/68 (4.41%)	3/97 (3.09%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Retinal exudates [A+]	8/104 (7.69%)	5/68 (7.35%)	4/97 (4.12%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Retinal haemorrhage [A+]	4/104 (3.85%)	6/68 (8.82%)	11/97 (11.34%)	5/57 (8.77%)	5/91 (5.49%)	2/52 (3.85%)
Retinal vascular disorder [A+]	6/104	7/68	10/97	2/57	0/91	2/52

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Visual acuity reduced [A]	2/104 (1.92%)	7/68 (10.29%)	10/97 (10.31%)	1/57 (1.75%)	7/91 (7.69%)	1/52 (1.92%)
Vitreous detachment [A]	2/104 (1.92%)	1/68 (1.47%)	7/97 (7.22%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vitreous floaters [A]	6/104 (5.77%)	0/68 (0%)	1/97 (1.03%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Gastrointestinal disorders						
Nausea [A]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Infections and infestations						
Influenza [A]	2/104 (1.92%)	0/68 (0%)	5/97 (5.15%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Nasopharyngitis [A]	8/104 (7.69%)	6/68 (8.82%)	10/97 (10.31%)	11/57 (19.3%)	4/91 (4.4%)	2/52 (3.85%)
Investigations						
Intraocular pressure increased [A]	9/104 (8.65%)	4/68 (5.88%)	14/97 (14.43%)	2/57 (3.51%)	2/91 (2.2%)	1/52 (1.92%)
Visual acuity tests abnormal [A]	0/104 (0%)	1/68 (1.47%)	5/97 (5.15%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Musculoskeletal and connective tissue disorders						
Arthralgia [A]	1/104 (0.96%)	5/68 (7.35%)	2/97 (2.06%)	1/57 (1.75%)	2/91 (2.2%)	0/52 (0%)
Nervous system disorders						
Headache [A]	7/104 (6.73%)	4/68 (5.88%)	4/97 (4.12%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Vascular disorders						
Hypertension [A*]	4/104 (3.85%)	3/68 (4.41%)	4/97 (4.12%)	4/57 (7.02%)	3/91 (3.3%)	2/52 (3.85%)

* Indicates events were collected by non-systematic methods.

A Term from vocabulary, MedDRA (14.1)

Limitations and Caveats

[Not specified]

More Information

Certain Agreements:

Principal Investigators are NOT employed by the organization sponsoring the study.

There IS an agreement between the Principal Investigator and the Sponsor (or its agents) that restricts the PI's rights to discuss or publish trial results after the trial is completed.

Publishing of result communication only after Bayer´s written approval. Manuscript to Bayer sixty days before public release. If no written Bayer comment within 60 days consider approval given. If multi-site study, principal investigator (PI) not do independently publish results before publication of the multi-site paper, but PI not restricted from 24 months from study to completion onwards.

Results Point of Contact:

Name/Official Title: Therapeutic Area Head
Organization: BAYER
Phone:
Email: clinical-trials-contact@bayerhealthcare.com

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ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
- Additions will be displayed in *green*.
- The yellow choices in the table indicate the study versions currently compared below. A yellow row indicates the study version being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: January 25, 2011 (v12)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	January 2011
Overall Status:	Active, not recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	January 25, 2011

Last Update Posted:	January 26, 2011 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5.

Change in the NEI-VFQ-25 total score from baseline
Week 24

6. Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT)
- Adults >= 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1

- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66424

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Marburg, Germany, 35037

Hungary

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Budapest, Hungary, 1133

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

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Nagoya, Aichi, Japan, 467-8602

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Japan, Osaka

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Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

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U.S. National Library of Medicine
ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
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- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
- Additions will be displayed in *green*.
- The yellow choices in the table indicate the study versions currently compared below. A yellow row indicates the study version being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

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Study NCT01012973
on Date: January 26, 2012 (v17)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	2009-010973-19 [EudraCT Number]

Study Status

Record Verification:	January 2012
Overall Status:	Active, not recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Actual]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Certification/Extension First Submitted:	January 26, 2012

Certification/Extension First Submitted that Met QC Criteria:	January 26, 2012
Certification/Extension First Posted:	January 30, 2012 [Estimate]
Last Update Submitted that Met QC Criteria:	January 26, 2012
Last Update Posted:	January 30, 2012 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment

Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	177 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Biological: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24

3. Absolute change from baseline in central retinal thickness, assessed by OCT
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4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5. Change in the NEI-VFQ-25 total score from baseline
   Week 24
6. Change in the EQ-5D score from baseline
   Week 24

Eligibility

Minimum Age:	18 Years
Maximum Age:	
Sex:	All
Gender Based:	
Accepts Healthy Volunteers:	No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness ≥ 250 µm on optical coherence tomography (OCT)
- Adults ≥ 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye

- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
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Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

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Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

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Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Marburg, Hessen, Germany, 35037

Germany, Niedersachsen

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Germany, Nordrhein-Westfalen

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Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66421

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

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Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Hungary

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Budapest, Hungary, 1133

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

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Roma, Italy, 00198

Torino, Italy, 10122

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Japan, Osaka

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Japan, Tokyo

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Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

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ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

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4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
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7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

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Study NCT01012973
on Date: January 30, 2014 (v22)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	2009-010973-19 [EudraCT Number]

Study Status

Record Verification:	January 2014
Overall Status:	Completed
Study Start:	October 2009
Primary Completion:	February 2011 [Actual]
Study Completion:	February 2012 [Actual]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Results First Submitted:	October 23, 2012
	October 23, 2012

Results First Submitted that Met QC Criteria:	
Results First Posted:	November 22, 2012 [Estimate]
Certification/Extension First Submitted:	January 26, 2012
Certification/Extension First Submitted that Met QC Criteria:	January 26, 2012
Certification/Extension First Posted:	January 30, 2012 [Estimate]
Last Update Submitted that Met QC Criteria:	January 30, 2014
Last Update Posted:	March 5, 2014 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	Sponsor
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion

CRVO
VEGF Trap-Eye
best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	177 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.	Biological: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Intravitreal injection. Weeks 0 to 20 of Aflibercept Injection every 4 weeks; Weeks 24 to 52 every 4 weeks PRN (pro re nata, on demand); plus additional on Week 60 and 68. Sham treatment Sham treatment. Weeks 0 to 52 sham treatment every 4 weeks; plus additional on Week 60 and 68.
Sham Comparator: Sham treatment Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the	Sham treatment Sham treatment. Weeks 0 to 52 sham treatment every 4 weeks; plus additional on Week 60 and 68.

Arms	Assigned Interventions
study retreatment criteria at Week 60 and 68.	

Outcome Measures

[See Results Section]

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness ≥ 250 µm on optical coherence tomography (OCT)
- Adults ≥ 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis

- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Marburg, Hessen, Germany, 35037

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66421

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Hungary

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Budapest, Hungary, 1133

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

Japan, Aichi

Nagoya, Aichi, Japan, 466-8560

Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

Suita, Osaka, Japan, 565-0871

Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of, Gyeonggido

Seongnam-si, Gyeonggido, Korea, Republic of, 463-707

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 137-701

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

IPDSharing

Plan to Share IPD:

References

Citations: [Study Results] Holz FG, Roider J, Ogura Y, Korobelnik JF, Simader C, Groetzbach G, Vitti R, Berliner AJ, Hiemeyer F, Beckmann K, Zeitz O, Sandbrink R. VEGF Trap-Eye for macular oedema secondary to central retinal vein occlusion: 6-month results of the phase III GALILEO study. Br J Ophthalmol. 2013 Mar;97(3):278-84. doi: 10.1136/bjophthalmol-2012-301504. Epub 2013 Jan 7.

Erratum in: Br J Ophthalmol. 2015 Dec;99(12):1746. PubMed 23298885

[Study Results] Korobelnik JF, Holz FG, Roider J, Ogura Y, Simader C, Schmidt-Erfurth U, Lorenz K, Honda M, Vitti R, Berliner AJ, Hiemeyer F, Stemper B, Zeitz O, Sandbrink R; GALILEO Study Group. Intravitreal Aflibercept Injection for Macular Edema Resulting from Central Retinal Vein Occlusion: One-Year Results of the Phase 3 GALILEO Study. Ophthalmology. 2014 Jan;121(1):202-208. doi: 10.1016/j.ophtha.2013.08.012. Epub 2013 Sep 29. PubMed 24084497

Links:

Available IPD/Information:

Study Results

Participant Flow

Reporting Groups

	Description
Aflibercept Injection First, Then Aflibercept Injection	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment First, Then Aflibercept Injection	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Overall Study

	Aflibercept Injection First, Then Aflibercept Injection	Sham Treatment First, Then Aflibercept Injection
Started	106	71
Participants Received Treatment	104 [1]	68 [1]
Fulfilled Requirements of FAS Population	103 [2]	68 [2]
Completed Week 24, From FAS	97	57
Completed Week 52, From FAS	91	52
Completed	90	52
Not Completed	16	19
Adverse Event	5	5
Lack of Efficacy	0	5
Lost to Follow-up	1	0
(Overseas travel - indefinite period)	1	0
Increase in vis. acuity, never injected	0	1
Protocol Violation	5	2
Withdrawal by Subject	4	6

[1] Safety Population: Participants received treatment

[2] Full Analysis Set (FAS) Population: Participants received treatment with post baseline measurements

Baseline Characteristics

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every

	4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Baseline Measures

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Overall Number of Participants		104	68	172
Age, Continuous Mean (Standard Deviation) Unit of measure: Years	Number Analyzed	104 Participants	68 Participants	172 Participants
		60.0 (12.3)	63.8 (13.3)	61.5 (12.8)
Sex: Female, Male Measure type: Count of Participants Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
	Female	45 43.27%	31 45.59%	76 44.19%
	Male	59 56.73%	37 54.41%	96 55.81%
Ethnicity (NIH/OMB) Measure type: Count of Participants Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
	Hispanic or Latino	4 3.85%	1 1.47%	5 2.91%
	Not Hispanic or Latino	100 96.15%	66 97.06%	166 96.51%
		0 0%	1 1.47%	1 0.58%

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
	Unknown or Not Reported			
Baseline Best Corrected Visual Acuity (BCVA) letter scores [1] Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		53.5 (15.7)	50.9 (15.4)	52.5 (15.6)
	[1]	Information retrieved from all baseline participants. Only participants with a ETDRS (Early Treatment Diabetic Retinopathy Study) Best Corrected Visual Acuity letter score of 73 to 25 (= Acuity of 20/40 to 20/320) in the study eye at 4 meters were included; a higher score represents better functioning.		
Number of participants with baseline retinal perfusion [1] Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
Perfused		90	54	144
Nonperfused		7	7	14
Indeterminate		7	7	14
	[1]	Retinal perfusion defined as less than 10 disc areas of capillary nonperfusion using fluorescein angiography (FA)		
Baseline Retinal Thickness by Optical Coherence Tomography (OCT) Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		682.78 (233.36)	638.66 (224.69)	665.34 (230.33)

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Baseline intraocular pressure Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		15.2 (2.8)	14.4 (2.7)	14.9 (2.8)
Number of participants with time since Central retinal vein occlusion (CRVO) diagnosis Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
>= 2 months		46	33	79
< 2 months		56	35	91
Missing		2	0	2
Baseline National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) total score [1]	Number Analyzed	104 Participants	68 Participants	172 Participants
		79.66 (13.06)	78.94 (14.00)	79.38 (13.40)
	[1]	The NEI VFQ-25 total score ranges from 0-100 with a score of 0 being the worst outcome and 100 being the best outcome. The NEI VFQ questionnaire is organized as a collection of subscales which are all scored from 0-100. To reach the overall composite score, each sub-scale score is averaged in order to give each sub-scale equal weight.		
European questionnaire 5 dimensions (EQ-5D) total score [1] Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		0.87 (0.15)	0.86 (0.16)	0.87 (0.15)

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Unit of measure: score on a scale				
	[1] The EQ-5D total score ranges from -0.594 to 1.000 with -0.594 being the worst.			
Race Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
Asian		26	15	41
White		75	49	124
Unknown or Not Reported		3	4	7

Outcome Measures

1. Primary Outcome Measure:

Measure Title	Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures
Measure Description	Defined study baseline range of Early Treatment Diabetic Retinopathy Study (ETDRS) Best Corrected Visual Acuity (BCVA) letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. Nominator = (Number of participants who maintained vision * 100); Denominator = Number of participants analyzed.
Time Frame	Baseline and Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures Measure Type: Number Unit of Measure: Percentage of participants	60.2	22.1

Statistical Analysis 1 for Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis of difference of Eylea minus Sham of 0 was tested. In the database close after Week 24, basis for primary efficacy evaluation, 56 Sham / 96 Eylea subjects were considered as week 24 completers.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	[Not specified]
	Method	Cochran-Mantel-Haenszel
	Comments	[Not specified]

Method of Estimation	Estimation Parameter	CMH adjusted difference
	Estimated Value	38.3
	Confidence Interval	(2-sided) 95% 24.4 to 52.1
	Estimation Comments	The estimate is calculated as Eylea minus Sham. A positive value shows Eylea showed a higher BCVA total score compared to Sham.

2. Secondary Outcome Measure:

Measure Title	Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF)
Measure Description	Defined study baseline range of ETDRS Best Corrected Visual Acuity letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. However, because this was assessed at the screening visit,

	subjects may have had a higher BCVA recorded at the baseline visit and would not have been excluded from the study.
Time Frame	Baseline and Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF) Measure Type: Mean (Standard Deviation) Unit of Measure: Letters correctly read	71.6 (17.1)	54.3 (20.2)

Statistical Analysis 1 for Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF)

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis was equality in change from baseline to Week 24 in BCVA total letter score between Eylea and Sham. If primary efficacy was successful, secondary efficacy endpoints were tested in a pre-specified fixed sequence testing procedure. Change in BCVA letter score was to be tested first in this sequence.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	As primary efficacy evaluation was significant, and this p-value was below significance level of two-sided <.05, the fixed sequence testing did continue with next secondary endpoint.
	Method	ANOVA
	Comments	ANOVA, adjusting for region and baseline BCVA category as fixed factors.

Method of Estimation	Estimation Parameter	Difference in Least square means
	Estimated Value	14.7
	Confidence Interval	(2-sided) 95% 10.8 to 18.7
	Estimation Comments	The difference is calculated as Eylea minus Sham. A positive value

		indicates Eylea showed a higher change in BCVA total score until week 24 compared to Sham.

3. Secondary Outcome Measure:

Measure Title	Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF
Measure Description	
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	67

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: microns	-448.58 (256.02)	-169.27 (224.72)

Statistical Analysis 1 for Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis was equality in change from baseline to Week 24 in central retinal thickness between Eylea and Sham. If primary efficacy was successful, secondary efficacy end points were to be tested in a pre-specified fixed sequence testing procedure. Change in central retinal thickness was to be tested at second place in this sequence.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	As fixed sequence testing did reject nullhypothesis of change from baseline in BCVA until week 24, and this p-value was below significance level of two-sided <.05, the fixed sequence testing did continue with next secondary endpoint.
	Method	ANCOVA
	Comments	ANCOVA, stratified by region and baseline BCVA category, baseline

		central retinal thickness added as covariate.

Method of Estimation	Estimation Parameter	Difference in Least square (LS) means
	Estimated Value	-239.42
	Confidence Interval	(2-sided) 95% -286.31 to -192.53
	Estimation Comments	The difference is calculated as Eylea minus Sham. A negative value indicates Eylea showed a higher reduction in change in central retinal thickness until week 24 compared to Sham.

4. Secondary Outcome Measure:

Measure Title	Percentage of Participants Who Developed Neovascularization During the First 24 Weeks
Measure Description	Formation of blood vessels in the anterior segment, optic disc, or elsewhere in the fundus up to Week 24
Time Frame	From baseline until Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-

	up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Percentage of Participants Who Developed Neovascularization During the First 24 Weeks Measure Type: Number Unit of Measure: Percentage of participants		
Any neovascularization	2.9	4.4
Anterior segment neovascularization	1.9	1.5
Neovascularization of the optic disc (NVD)	0.0	0.0
Neovascularization elsewhere in the fundus (NVE)	1.0	2.9

Statistical Analysis 1 for Percentage of Participants Who Developed Neovascularization During the First 24 Weeks

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Nullhypothesis of no difference in development of neovascularizations between Eylea and Sham group was tested. (Any neovascularization)
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	0.5947
	Comments	

		As fixed sequence testing did reject nullhypothesis of change from baseline in CRT until week 24, and this p-value was not below significance level of two-sided <.05, the fixed sequence testing did end with this evaluation.
	Method	Cochran-Mantel-Haenszel
	Comments	Cochrane-Mantel-Haenszel test, stratified by region and baseline BCVA category.

Method of Estimation	Estimation Parameter	CMH adjusted Difference
	Estimated Value	-1.5
	Confidence Interval	(2-sided) 95% -7.4 to 4.4
	Estimation Comments	[Not specified]

5. Secondary Outcome Measure:

Measure Title	Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF
Measure Description	The NEI VFQ-25 total score ranges from 0-100 with a score of 0 being the worst outcome and 100 being the best outcome. The NEI VFQ questionnaire is organized as a collection of subscales which are all scored from 0-100. To reach the overall composite score, each sub-scale score is averaged in order to give each sub-scale equal weight
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	96	65
Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: Scores on a scale	7.46 (9.55)	3.55 (9.74)

Statistical Analysis 1 for Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	[Not specified]
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Method of Estimation	Estimation Parameter	Difference in LS means
	Estimated Value	4.2
	Confidence Interval	(2-sided) 95% 1.7 to 6.8
	Estimation Comments	As the fixed sequence of secondary endpoints stopped with proportion of neovascularizations developed until week 24, 95% confidence interval is only of descriptive nature.

6. Secondary Outcome Measure:

Measure Title	Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF
Measure Description	EQ-5D is a quality of life questionnaire based on a scale from -0.594 (worst) to 1.00 (best).
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and

	depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	95	64
Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: Scores on a scale	0.029 (0.139)	-0.002 (0.195)

Statistical Analysis 1 for Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	[Not specified]
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Method of Estimation	Estimation Parameter	Difference in LS Means
	Estimated Value	0.044
	Confidence Interval	(2-sided) 95% -0.002 to 0.09
	Estimation Comments	As the fixed sequence of secondary endpoints stopped with proportion of neovascularizations developed until week 24, 95% confidence interval is only of descriptive nature.

Reported Adverse Events

Time Frame	[Not specified]
	[Not specified]

Adverse Event Reporting Description	

Reporting Groups

	Description
Aflibercept Injection (Until Week 20)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20. Participants were observed until Week 24. Participants in the safety population were at risk.
Sham Treatment (Until Week 20)	Participants received sham treatment administered every 4 weeks from Day 1 through Week 20. Participants were observed until Week 24. Participants in the safety population were at risk.
Aflibercept Injection (Until Week 48)	Participants who continued the study drug until Week 24 received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Participants were observed from Week 24 until Week 52. Participants in the safety population that completed Week 24 were at risk.
Sham Treatment (Until Week 48)	Participants who continued the study drug until Week 24 received sham treatment administered every 4 weeks from Week 24 to Week 48. Participants were observed from Week 24 until Week 52. Participants in the safety population that completed Week 24 were at risk.
Aflibercept Injection Continued (Until Week 68)	Participants on IAI who continued the study drug until Week 52, received 2 mg dose of IAI depending on the study retreatment criteria at Week 52, 60 and 68. Participants were observed starting from Week 52. Participants in the safety population that completed Week 52 were at risk.
Sham Treatment Then Aflibercept Injection (Until Week 68)	Participants on sham treatment switched to IAI, received a 2 mg dose of IAI at Week 52 and depending on the study retreatment criteria at Week

	60 and 68. Participants were observed starting from Week 52. Participants in the safety population that completed Week 52 were at risk.

All-Cause Mortality

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)
Total	/	/	/	/	/	/

Serious Adverse Events

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)
Total	8/104 (7.69%)	8/68 (11.76%	14/97 (14.43%	7/57 (12.28%	4/91 (4.4%)	3/52 (5.77%)
Cardiac disorders						
Aortic valve incompetence [A-]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Cardiac failure [A-]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Coronary artery stenosis [A-]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Diastolic dysfunction [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Mitral valve incompetence [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Eye disorders						
Blindness unilateral [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Glaucoma [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Iris neovascularisation [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular fibrosis [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular ischaemia [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular oedema [A*]	0/104 (0%)	2/68 (2.94%)	4/97 (4.12%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Retinal vein occlusion [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Visual acuity reduced [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	0/57 (0%)	2/91 (2.2%)	0/52 (0%)
Vitreous detachment [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vitreous haemorrhage [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Gastrointestinal disorders						

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Diverticular perforation [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Hepatobiliary disorders						
Hepatic function abnormal [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Infections and infestations						
Furuncle [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Gastroenteritis [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Pneumonia [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Vestibular neuronitis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Injury, poisoning and procedural complications						
Fall [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Femur fracture [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Hand fracture [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Humerus fracture [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Radius fracture [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Spinal compression fracture [A*]						

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Musculoskeletal and connective tissue disorders						
Intervertebral disc protrusion [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Spinal column stenosis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)						
Breast cancer [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Oropharyngeal cancer stage unspecified [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Nervous system disorders						
Paraesthesia [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Syncope [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	2/57 (3.51%)	0/91 (0%)	0/52 (0%)
Transient ischaemic attack [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Respiratory, thoracic and mediastinal disorders						
Dyspnoea [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Laryngeal granuloma [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Pulmonary hypertension [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Surgical and medical procedures						
Ischaemic heart disease prophylaxis [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vascular disorders						
Circulatory collapse [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)

* Indicates events were collected by non-systematic methods.

A Term from vocabulary, MedDRA (14.1)

Other Adverse Events

Frequency Threshold Above Which Other Adverse Events are Reported: 5%

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)
Total	52/104 (50%)	44/68 (64.71%)	66/97 (68.04%)	30/57 (52.63%)	38/91 (41.76%)	19/52 (36.54%)
Blood and lymphatic system disorders						
Anaemia [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Eye disorders						
Conjunctival haemorrhage [A*]						

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	10/104 (9.62%)	3/68 (4.41%)	3/97 (3.09%)	0/57 (0%)	9/91 (9.89%)	3/52 (5.77%)
Eye irritation [A*]	3/104 (2.88%)	7/68 (10.29%)	4/97 (4.12%)	1/57 (1.75%)	1/91 (1.1%)	2/52 (3.85%)
Eye pain [A*]	12/104 (11.54%)	3/68 (4.41%)	6/97 (6.19%)	2/57 (3.51%)	1/91 (1.1%)	0/52 (0%)
Foreign body sensation in eyes [A*]	6/104 (5.77%)	5/68 (7.35%)	2/97 (2.06%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Lacrimation increased [A*]	3/104 (2.88%)	4/68 (5.88%)	3/97 (3.09%)	4/57 (7.02%)	1/91 (1.1%)	2/52 (3.85%)
Macular fibrosis [A*]	1/104 (0.96%)	1/68 (1.47%)	5/97 (5.15%)	4/57 (7.02%)	0/91 (0%)	3/52 (5.77%)
Macular ischaemia [A*]	7/104 (6.73%)	5/68 (7.35%)	3/97 (3.09%)	1/57 (1.75%)	0/91 (0%)	1/52 (1.92%)
Macular oedema [A*]	2/104 (1.92%)	9/68 (13.24%)	30/97 (30.93%)	7/57 (12.28%)	17/91 (18.68%)	2/52 (3.85%)
Ocular hyperaemia [A*]	5/104 (4.81%)	4/68 (5.88%)	2/97 (2.06%)	1/57 (1.75%)	4/91 (4.4%)	1/52 (1.92%)
Optic disc vascular disorder [A*]	5/104 (4.81%)	3/68 (4.41%)	3/97 (3.09%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Retinal exudates [A*]	8/104 (7.69%)	5/68 (7.35%)	4/97 (4.12%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Retinal haemorrhage [A*]	4/104 (3.85%)	6/68 (8.82%)	11/97 (11.34%)	5/57 (8.77%)	5/91 (5.49%)	2/52 (3.85%)
Retinal vascular disorder [A*]	6/104 (5.77%)	7/68 (10.29%)	10/97 (10.31%)	2/57 (3.51%)	0/91 (0%)	2/52 (3.85%)

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	2/104 (1.92%)	7/68 (10.29%)	10/97 (10.31%)	1/57 (1.75%)	7/91 (7.69%)	1/52 (1.92%)
Vitreous detachment [A*]	2/104 (1.92%)	1/68 (1.47%)	7/97 (7.22%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vitreous floaters [A*]	6/104 (5.77%)	0/68 (0%)	1/97 (1.03%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Gastrointestinal disorders						
Nausea [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Infections and infestations						
Influenza [A*]	2/104 (1.92%)	0/68 (0%)	5/97 (5.15%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Nasopharyngitis [A*]	8/104 (7.69%)	6/68 (8.82%)	10/97 (10.31%)	11/57 (19.3%)	4/91 (4.4%)	2/52 (3.85%)
Investigations						
Intraocular pressure increased [A*]	9/104 (8.65%)	4/68 (5.88%)	14/97 (14.43%)	2/57 (3.51%)	2/91 (2.2%)	1/52 (1.92%)
Visual acuity tests abnormal [A*]	0/104 (0%)	1/68 (1.47%)	5/97 (5.15%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Musculoskeletal and connective tissue disorders						
Arthralgia [A*]	1/104 (0.96%)	5/68 (7.35%)	2/97 (2.06%)	1/57 (1.75%)	2/91 (2.2%)	0/52 (0%)
Nervous system disorders						
Headache [A*]	7/104 (6.73%)	4/68 (5.88%)	4/97 (4.12%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Vascular disorders						

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Hypertension [A-]	4/104 (3.85%)	3/68 (4.41%)	4/97 (4.12%)	4/57 (7.02%)	3/91 (3.3%)	2/52 (3.85%)

* Indicates events were collected by non-systematic methods.

A Term from vocabulary, MedDRA (14.1)

Limitations and Caveats

[Not specified]

More Information

Certain Agreements:

Principal Investigators are NOT employed by the organization sponsoring the study.

There IS an agreement between the Principal Investigator and the Sponsor (or its agents) that restricts the PI's rights to discuss or publish trial results after the trial is completed.

Publishing of result communication only after Bayer´s written approval. Manuscript to Bayer sixty days before public release. If no written Bayer comment within 60 days consider approval given. If multi-site study, principal investigator (PI) not do independently publish results before publication of the multi-site paper, but PI not restricted from 24 months from study to completion onwards.

Results Point of Contact:

Name/Official Title: Therapeutic Area Head
Organization: BAYER
Phone:
Email: clinical-trials-contact@bayerhealthcare.com

Scroll up to access the controls Scroll to the Study top

U.S. National Library of Medicine
ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
- Additions will be displayed in *green*.
- The yellow choices in the table indicate the study versions currently compared below. A yellow row indicates the study version being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare

Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: February 9, 2010 (v3)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	February 2010
Overall Status:	Recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	August 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	February 9, 2010

Last Update Posted:	February 10, 2010 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; weeks 52 to 100 safety follow-up.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; weeks 52 to 100 safety follow-up.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5. Change in the NEI-VFQ-25 total score from baseline
   Week 24
6. Change in the EQ-5D score from baseline
   Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT).
- Adults >= 18 years.
- early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye.

Exclusion Criteria:

- Previous treatment with anti-angiogenic drugs in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.)
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Central Contact: Bayer Clinical Trials Contact
Email: clinical-trials-contact@bayerhealthcare.com

Study Officials:

Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

[Not yet recruiting]
Chatswood, New South Wales, Australia, 2067

[Recruiting]
Sydney, New South Wales, Australia, 2000

[Not yet recruiting]
Westmead, New South Wales, Australia, 2145

Australia, Victoria

[Recruiting]
East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

[Recruiting]
Nedlands, Western Australia, Australia, 6009

Australia

[Recruiting]
Parramatta, Australia, 2150

Austria, Oberösterreich

[Not yet recruiting]
Linz, Oberösterreich, Austria, 4020

Austria, Tirol

[Not yet recruiting]
Innsbruck, Tirol, Austria, 6020

Austria

[Recruiting]
Linz, Austria, 4021

[Recruiting]
Wien, Austria, 1090

France, Cedex 12

[Not yet recruiting]
Paris, Cedex 12, France, 75557

France

[Not yet recruiting]
Bordeaux, France, 33000

[Not yet recruiting]
Dijon Cedex, France, BP 1542-21

[Not yet recruiting]
Marseille, France, 13008

[Not yet recruiting]
Nantes Cedex, France, 44035

[Not yet recruiting]
Paris, France, 75015

Germany, Baden-Württemberg

[Recruiting]
Freiburg, Baden-Württemberg, Germany, 79106

[Not yet recruiting]
Heidelberg, Baden-Württemberg, Germany, 69120

[Recruiting]
Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

[Recruiting]
München, Bayern, Germany, 81675

[Recruiting]
Regensburg, Bayern, Germany, 93053

Germany, Hessen

[Recruiting]
Darmstadt, Hessen, Germany, 64276

[Not yet recruiting]
Marburg, Hessen, Germany, 35043

Germany, Niedersachsen

[Not yet recruiting]
Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

[Not yet recruiting]
Aachen, Nordrhein-Westfalen, Germany, 52074

[Recruiting]
Bonn, Nordrhein-Westfalen, Germany, 53105

[Not yet recruiting]
Essen, Nordrhein-Westfalen, Germany, 45147

[Not yet recruiting]
Köln, Nordrhein-Westfalen, Germany, 50931

Germany, Rheinland-Pfalz

[Recruiting]
Ludwigshafen, Rheinland-Pfalz, Germany, 67063

[Recruiting]
Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

[Not yet recruiting]
Homburg, Saarland, Germany, 66421

Germany, Sachsen

[Not yet recruiting]
Chemnitz, Sachsen, Germany, 09116

[Not yet recruiting]
Dresden, Sachsen, Germany, 01067

[Not yet recruiting]
Dresden, Sachsen, Germany, 01307

[Recruiting]
Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

[Not yet recruiting]
Kiel, Schleswig-Holstein, Germany, 24105

[Not yet recruiting]
Lübeck, Schleswig-Holstein, Germany, 23538

Germany

[Not yet recruiting]
Hamburg, Germany, 20251

Hungary

[Not yet recruiting]
Budapest, Hungary, 1036

[Not yet recruiting]
Budapest, Hungary, 1089

[Not yet recruiting]
Budapest, Hungary, 1106

[Recruiting]
Debrecen, Hungary, 4032

[Not yet recruiting]
Veszprem, Hungary, 8200

Italy

[Not yet recruiting]
Ancona, Italy, 60126

[Not yet recruiting]
Bari, Italy, 70124

[Not yet recruiting]
Catania, Italy, 95123

[Not yet recruiting]
Firenze, Italy, 50139

[Not yet recruiting]
Milano, Italy, 20122

[Not yet recruiting]
Milano, Italy, 20132

[Not yet recruiting]
Milano, Italy, 20157

[Not yet recruiting]
Padova, Italy, 35128

[Not yet recruiting]
Roma, Italy, 00133

[Not yet recruiting]
Roma, Italy, 00185

[Not yet recruiting]
Torino, Italy, 10149

Japan, Aichi

[Not yet recruiting]
Nagoya, Aichi, Japan, 466-8560

[Not yet recruiting]
Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

[Recruiting]
Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

[Not yet recruiting]
Suita, Osaka, Japan, 565-0871

Japan, Tokyo

[Not yet recruiting]
Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

[Not yet recruiting]
Kyoto, Japan, 606-8507

Korea, Republic of

[Recruiting]
Ask Contact, Korea, Republic of

[Not yet recruiting]
Incheon, Korea, Republic of, 405-760

[Recruiting]
Kungki-do, Korea, Republic of, 463-707

[Recruiting]
Seoul, Korea, Republic of, 110 744

[Not yet recruiting]
Seoul, Korea, Republic of, 110-744

[Recruiting]
Seoul, Korea, Republic of, 138-736

Latvia

[Not yet recruiting]
Riga, Latvia, 1009

[Recruiting]
Riga, Latvia, LV-1002

Singapore

[Not yet recruiting]
Singapore, Singapore, 119074

[Not yet recruiting]
Singapore, Singapore, 168751

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U.S. National Library of Medicine
ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
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- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
- Additions will be displayed in *green*.
- The yellow choices in the table indicate the study versions currently compared below. A yellow row indicates the study version being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

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Study NCT01012973
on Date: February 20, 2012 (v18)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	2009-010973-19 [EudraCT Number]

Study Status

Record Verification:	February 2012
Overall Status:	Completed
Study Start:	October 2009
Primary Completion:	February 2011 [Actual]
Study Completion:	February 2012 [Actual]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Certification/Extension First Submitted:	January 26, 2012

Certification/Extension First Submitted that Met QC Criteria:	January 26, 2012
Certification/Extension First Posted:	January 30, 2012 [Estimate]
Last Update Submitted that Met QC Criteria:	February 20, 2012
Last Update Posted:	February 23, 2012 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment

Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	177 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Biological: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24

3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5. Change in the NEI-VFQ-25 total score from baseline
   Week 24
6. Change in the EQ-5D score from baseline
   Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness ≥ 250 µm on optical coherence tomography (OCT)
- Adults ≥ 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye

- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Marburg, Hessen, Germany, 35037

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66421

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Hungary

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Budapest, Hungary, 1133

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

Japan, Aichi

Nagoya, Aichi, Japan, 466-8560

Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

Suita, Osaka, Japan, 565-0871

Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

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History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

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5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
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8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
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16	○	○	November 29, 2011	Study Status and Study Identification
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22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

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Study NCT01012973
on Date: March 16, 2010 (v4)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19 GALILEO

Study Status

Record Verification:	March 2010
Overall Status:	Recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	August 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
	March 16, 2010

Last Update Submitted that Met QC Criteria:	
Last Update Posted:	March 17, 2010 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized

Enrollment: 165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; weeks 52 to 100 safety follow-up.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; weeks 52 to 100 safety follow-up.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5. Change in the NEI-VFQ-25 total score from baseline
   Week 24
6. Change in the EQ-5D score from baseline
   Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT).
- Adults >= 18 years.
- early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye.

Exclusion Criteria:

- Previous treatment with anti-angiogenic drugs in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.)
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Central Contact: Bayer Clinical Trials Contact
Email: clinical-trials-contact@bayerhealthcare.com

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

[Not yet recruiting]
Chatswood, New South Wales, Australia, 2067

[Recruiting]
Sydney, New South Wales, Australia, 2000

[Recruiting]
Westmead, New South Wales, Australia, 2145

Australia, Victoria

[Recruiting]
East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

[Recruiting]
Nedlands, Western Australia, Australia, 6009

Australia

[Recruiting]
Parramatta, Australia, 2150

Austria, Oberösterreich

[Not yet recruiting]
Linz, Oberösterreich, Austria, 4020

Austria, Tirol

[Not yet recruiting]
Innsbruck, Tirol, Austria, 6020

Austria

[Recruiting]
Linz, Austria, 4021

[Recruiting]
Wien, Austria, 1090

France, Cedex 12

[Not yet recruiting]
Paris, Cedex 12, France, 75557

France

[Not yet recruiting]
Bordeaux, France, 33000

[Not yet recruiting]
Dijon Cedex, France, BP 1542-21

[Not yet recruiting]
Marseille, France, 13008

[Not yet recruiting]
Nantes Cedex, France, 44035

[Not yet recruiting]
Paris, France, 75015

Germany, Baden-Württemberg

[Recruiting]
Freiburg, Baden-Württemberg, Germany, 79106

[Recruiting]
Heidelberg, Baden-Württemberg, Germany, 69120

[Recruiting]
Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

[Recruiting]
München, Bayern, Germany, 81675

[Recruiting]
Regensburg, Bayern, Germany, 93053

Germany, Hessen

[Recruiting]
Darmstadt, Hessen, Germany, 64276

[Not yet recruiting]
Marburg, Hessen, Germany, 35043

Germany, Niedersachsen

[Recruiting]
Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

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Aachen, Nordrhein-Westfalen, Germany, 52074

[Recruiting]
Bonn, Nordrhein-Westfalen, Germany, 53105

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Essen, Nordrhein-Westfalen, Germany, 45147

[Not yet recruiting]
Köln, Nordrhein-Westfalen, Germany, 50931

Germany, Rheinland-Pfalz

[Recruiting]
Ludwigshafen, Rheinland-Pfalz, Germany, 67063

[Recruiting]
Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

[Not yet recruiting]
Homburg, Saarland, Germany, 66421

Germany, Sachsen

[Not yet recruiting]
Chemnitz, Sachsen, Germany, 09116

[Not yet recruiting]
Dresden, Sachsen, Germany, 01067

[Not yet recruiting]
Dresden, Sachsen, Germany, 01307

[Recruiting]
Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

[Not yet recruiting]
Kiel, Schleswig-Holstein, Germany, 24105

[Not yet recruiting]
Lübeck, Schleswig-Holstein, Germany, 23538

Germany

[Not yet recruiting]
Hamburg, Germany, 20251

Hungary

[Not yet recruiting]
Budapest, Hungary, 1036

[Recruiting]
Budapest, Hungary, 1089

[Recruiting]
Budapest, Hungary, 1106

[Recruiting]
Debrecen, Hungary, 4032

[Not yet recruiting]
Veszprem, Hungary, 8200

Italy

[Not yet recruiting]
Ancona, Italy, 60126

[Not yet recruiting]
Bari, Italy, 70124

[Not yet recruiting]
Catania, Italy, 95123

[Not yet recruiting]
Firenze, Italy, 50139

[Not yet recruiting]
Milano, Italy, 20122

[Not yet recruiting]
Milano, Italy, 20132

[Not yet recruiting]
Milano, Italy, 20157

[Not yet recruiting]
Padova, Italy, 35128

[Not yet recruiting]
Roma, Italy, 00133

[Not yet recruiting]
Roma, Italy, 00185

[Not yet recruiting]
Torino, Italy, 10149

Japan, Aichi

[Recruiting]
Nagoya, Aichi, Japan, 466-8560

[Not yet recruiting]
Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

[Recruiting]
Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

[Recruiting]
Suita, Osaka, Japan, 565-0871

Japan, Tokyo

[Not yet recruiting]
Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

[Not yet recruiting]
Kyoto, Japan, 606-8507

Korea, Republic of

[Not yet recruiting]
Incheon, Korea, Republic of, 405-760

[Recruiting]
Kungki-do, Korea, Republic of, 463-707

[Recruiting]
Seongsanno, Korea, Republic of

[Recruiting]
Seoul, Korea, Republic of, 110 744

[Not yet recruiting]
Seoul, Korea, Republic of, 110-744

[Recruiting]
Seoul, Korea, Republic of, 138-736

Latvia

[Not yet recruiting]
Riga, Latvia, 1009

[Recruiting]
Riga, Latvia, LV-1002

Singapore

[Not yet recruiting]
Singapore, Singapore, 119074

[Recruiting]
Singapore, Singapore, 168751

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References

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ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

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Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

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Study NCT01012973
on Date: April 8, 2011 (v13)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	April 2011
Overall Status:	Active, not recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Actual]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	April 8, 2011

Last Update Posted:	April 11, 2011 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	177 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5.

Change in the NEI-VFQ-25 total score from baseline
Week 24

6. Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT)
- Adults >= 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1

- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66424

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Marburg, Germany, 35037

Hungary

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Budapest, Hungary, 1133

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

Japan, Aichi

Nagoya, Aichi, Japan, 466-8560

Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

Suita, Osaka, Japan, 565-0871

Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

IPDSharing

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U.S. National Library of Medicine
ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

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- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
- Additions will be displayed in *green*.
- The yellow choices in the table indicate the study versions currently compared below. A yellow row indicates the study version being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

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5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
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22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: April 16, 2010 (v5)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	April 2010
Overall Status:	Recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	August 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	April 16, 2010

Last Update Posted:	April 19, 2010 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; weeks 52 to 100 safety follow-up.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; weeks 52 to 100 safety follow-up.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5. Change in the NEI-VFQ-25 total score from baseline
   Week 24
6. Change in the EQ-5D score from baseline
   Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:	
Sex:	All
Gender Based:	
Accepts Healthy Volunteers:	No
Criteria:	Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT).
- Adults >= 18 years.
- early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye.

Exclusion Criteria:

- Previous treatment with anti-angiogenic drugs in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.)
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Central Contact:	Bayer Clinical Trials Contact Email: clinical-trials-contact@bayerhealthcare.com
Study Officials:	

Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

[Not yet recruiting]
Chatswood, New South Wales, Australia, 2067

[Recruiting]
Sydney, New South Wales, Australia, 2000

[Recruiting]
Westmead, New South Wales, Australia, 2145

Australia, Victoria

[Recruiting]
East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

[Recruiting]
Nedlands, Western Australia, Australia, 6009

Australia

[Recruiting]
Parramatta, Australia, 2150

Austria, Oberösterreich

[Not yet recruiting]
Linz, Oberösterreich, Austria, 4020

Austria, Tirol

[Not yet recruiting]
Innsbruck, Tirol, Austria, 6020

Austria

[Recruiting]
Linz, Austria, 4021

[Recruiting]
Wien, Austria, 1090

France, Cedex 12

[Not yet recruiting]
Paris, Cedex 12, France, 75557

France

[Not yet recruiting]
Bordeaux, France, 33000

[Not yet recruiting]
Dijon Cedex, France, BP 1542-21

[Not yet recruiting]
Marseille, France, 13008

[Not yet recruiting]
Nantes Cedex, France, 44035

[Not yet recruiting]
Paris, France, 75015

Germany, Baden-Württemberg

[Recruiting]
Freiburg, Baden-Württemberg, Germany, 79106

[Recruiting]
Heidelberg, Baden-Württemberg, Germany, 69120

[Recruiting]
Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

[Recruiting]
München, Bayern, Germany, 81675

[Recruiting]
Regensburg, Bayern, Germany, 93053

Germany, Hessen

[Recruiting]
Darmstadt, Hessen, Germany, 64276

[Not yet recruiting]
Marburg, Hessen, Germany, 35043

Germany, Niedersachsen

[Recruiting]
Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

[Recruiting]
Aachen, Nordrhein-Westfalen, Germany, 52074

[Recruiting]
Bonn, Nordrhein-Westfalen, Germany, 53105

[Not yet recruiting]
Essen, Nordrhein-Westfalen, Germany, 45147

[Recruiting]
Köln, Nordrhein-Westfalen, Germany, 50931

Germany, Rheinland-Pfalz

[Recruiting]
Ludwigshafen, Rheinland-Pfalz, Germany, 67063

[Recruiting]
Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

[Recruiting]
Homburg, Saarland, Germany, 66421

Germany, Sachsen

[Recruiting]
Chemnitz, Sachsen, Germany, 09116

[Not yet recruiting]
Dresden, Sachsen, Germany, 01067

[Not yet recruiting]
Dresden, Sachsen, Germany, 01307

[Recruiting]
Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

[Not yet recruiting]
Kiel, Schleswig-Holstein, Germany, 24105

[Not yet recruiting]
Lübeck, Schleswig-Holstein, Germany, 23538

Germany

[Not yet recruiting]
Hamburg, Germany, 20251

Hungary

[Recruiting]
Budapest, Hungary, 1036

[Recruiting]
Budapest, Hungary, 1089

[Recruiting]
Budapest, Hungary, 1106

[Recruiting]
Debrecen, Hungary, 4032

[Suspended]
Veszprem, Hungary, 8200

[Not yet recruiting]
Zalaegerszeg, Hungary, 8900

Italy

[Not yet recruiting]
Ancona, Italy, 60126

[Not yet recruiting]
Bari, Italy, 70124

[Not yet recruiting]
Catania, Italy, 95123

[Not yet recruiting]
Firenze, Italy, 50139

[Recruiting]
Milano, Italy, 20122

[Not yet recruiting]
Milano, Italy, 20132

[Not yet recruiting]
Milano, Italy, 20157

[Not yet recruiting]
Padova, Italy, 35128

[Not yet recruiting]
Roma, Italy, 00133

[Not yet recruiting]
Roma, Italy, 00185

[Not yet recruiting]
Torino, Italy, 10149

Japan, Aichi

[Recruiting]
Nagoya, Aichi, Japan, 466-8560

[Recruiting]
Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

[Recruiting]
Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

[Recruiting]
Suita, Osaka, Japan, 565-0871

Japan, Tokyo

[Not yet recruiting]
Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

[Recruiting]
Kyoto, Japan, 606-8507

Korea, Republic of

[Recruiting]
ask Contact, Korea, Republic of

[Not yet recruiting]
Incheon, Korea, Republic of, 405-760

[Recruiting]
Kungki-do, Korea, Republic of, 463-707

[Recruiting]
Seoul, Korea, Republic of, 110 744

[Recruiting]
Seoul, Korea, Republic of, 110-744

[Recruiting]
Seoul, Korea, Republic of, 138-736

Latvia

[Not yet recruiting]
Riga, Latvia, 1009

[Recruiting]
Riga, Latvia, LV-1002

Singapore

[Recruiting]
Singapore, Singapore, 119074

[Recruiting]
Singapore, Singapore, 168751

IPDSharing

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U.S. National Library of Medicine
ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

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Compare | Comparison Format: (•) Merged ( ) Side-by-Side

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Study NCT01012973
on Date: June 23, 2011 (v14)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	June 2011
Overall Status:	Active, not recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Actual]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	June 23, 2011

Last Update Posted:	June 27, 2011 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	177 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Biological: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5.

Change in the NEI-VFQ-25 total score from baseline
Week 24

6. Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness ≥ 250 µm on optical coherence tomography (OCT)
- Adults ≥ 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1

- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations:

Australia, New South Wales

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66421

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Marburg, Germany, 35037

Hungary

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Budapest, Hungary, 1133

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

Japan, Aichi

Nagoya, Aichi, Japan, 466-8560

Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

Suita, Osaka, Japan, 565-0871

Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

IPDSharing

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Citations:

Links:

Available IPD/Information:

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U.S. National Library of Medicine
ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
- Additions will be displayed in *green*.
- The yellow choices in the table indicate the study versions currently compared below. A yellow row indicates the study version being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: July 22, 2010 (v6)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	July 2010
Overall Status:	Recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	July 22, 2010

Last Update Posted:	July 23, 2010 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5.

Change in the NEI-VFQ-25 total score from baseline
Week 24

6. Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT)
- Adults >= 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1

- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Central Contact: Bayer Clinical Trials Contact
Email: clinical-trials-contact@bayerhealthcare.com

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

[Recruiting]
Chatswood, New South Wales, Australia, 2067

[Recruiting]
Parramatta, New South Wales, Australia, 2150

[Recruiting]
Sydney, New South Wales, Australia, 2000

[Recruiting]
Westmead, New South Wales, Australia, 2145

Australia, Victoria

[Recruiting]
East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

[Recruiting]
Nedlands, Western Australia, Australia, 6009

Austria

[Terminated]
Innsbruck, Austria, 6020

[Recruiting]
Linz, Austria, 4021

[Recruiting]
Wien, Austria, 1090

France, Cedex 12

[Not yet recruiting]
Paris, Cedex 12, France, 75557

France, Cedex 1

[Recruiting]
Nantes, Cedex 1, France, 44093

France

[Recruiting]
Bordeaux, France, 33000

[Recruiting]
Dijon, France, 21033

[Recruiting]
Marseille, France, 13008

[Recruiting]
Paris, France, 75015

Germany, Baden-Württemberg

[Recruiting]
Freiburg, Baden-Württemberg, Germany, 79106

[Recruiting]
Heidelberg, Baden-Württemberg, Germany, 69120

[Recruiting]
Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

[Recruiting]
München, Bayern, Germany, 81675

[Recruiting]
Regensburg, Bayern, Germany, 93053

Germany, Hessen

[Recruiting]
Darmstadt, Hessen, Germany, 64297

[Not yet recruiting]
Frankfurt, Hessen, Germany, 60596

Germany, Niedersachsen

[Recruiting]
Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

[Recruiting]
Aachen, Nordrhein-Westfalen, Germany, 52074

[Recruiting]
Bonn, Nordrhein-Westfalen, Germany, 53105

[Not yet recruiting]
Essen, Nordrhein-Westfalen, Germany, 45122

[Recruiting]
Köln, Nordrhein-Westfalen, Germany, 50924

[Not yet recruiting]
Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

[Recruiting]
Ludwigshafen, Rheinland-Pfalz, Germany, 67063

[Recruiting]
Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

[Recruiting]
Homburg, Saarland, Germany, 66424

Germany, Sachsen

[Recruiting]
Chemnitz, Sachsen, Germany, 09116

[Not yet recruiting]
Dresden, Sachsen, Germany, 01307

[Recruiting]
Dresden, Sachsen, Germany, 06067

[Recruiting]
Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

[Not yet recruiting]
Kiel, Schleswig-Holstein, Germany, 24105

[Not yet recruiting]
Lübeck, Schleswig-Holstein, Germany, 23538

Germany

[Not yet recruiting]
Berlin, Germany, 13353

[Recruiting]
Hamburg, Germany, 20251

[Recruiting]
Marburg, Germany, 35037

Hungary

[Recruiting]
Budapest, Hungary, 1036

[Recruiting]
Budapest, Hungary, 1089

[Recruiting]
Budapest, Hungary, 1106

[Recruiting]
Debrecen, Hungary, 4032

[Terminated]
Veszprem, Hungary, 8200

[Not yet recruiting]
Zalaegerszeg, Hungary, H-8900

Italy

[Recruiting]
Ancona, Italy, 60126

[Recruiting]
Bari, Italy, 70124

[Not yet recruiting]
Catania, Italy, 95123

[Recruiting]
Firenze, Italy, 50134

[Recruiting]
Milano, Italy, 20122

[Recruiting]
Milano, Italy, 20132

[Not yet recruiting]
Milano, Italy, 20157

[Terminated]
Padova, Italy, 35128

[Recruiting]
Roma, Italy, 00133

[Recruiting]
Roma, Italy, 00198

[Not yet recruiting]
Torino, Italy, 10122

Japan, Aichi

[Recruiting]
Nagoya, Aichi, Japan, 466-8560

[Recruiting]
Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

[Recruiting]
Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

[Recruiting]
Suita, Osaka, Japan, 565-0871

Japan, Tokyo

[Recruiting]
Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

[Recruiting]
Kyoto, Japan, 606-8507

Korea, Republic of

[Recruiting]
Incheon, Korea, Republic of, 405-760

[Recruiting]
Kungki-do, Korea, Republic of, 463-707

[Recruiting]
Seoul, Korea, Republic of, 110 744

[Recruiting]
Seoul, Korea, Republic of, 110-744

[Recruiting]
Seoul, Korea, Republic of, 138-736

[Recruiting]
Seoul, Korea, Republic of

Latvia

[Recruiting]
Riga, Latvia, 1009

[Recruiting]
Riga, Latvia, LV-1002

Singapore

[Recruiting]
Singapore, Singapore, 119074

[Recruiting]
Singapore, Singapore, 168751

IPDSharing

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References

Citations:

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U.S. National Library of Medicine
ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

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3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: August 25, 2010 (v7)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	August 2010
Overall Status:	Recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	August 25, 2010

Last Update Posted:	August 26, 2010 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5.

Change in the NEI-VFQ-25 total score from baseline
Week 24

6. Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT)
- Adults >= 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1

- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Central Contact: Bayer Clinical Trials Contact
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Westmead, New South Wales, Australia, 2145

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Nedlands, Western Australia, Australia, 6009

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[Active, not recruiting]
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[Active, not recruiting]
Wien, Austria, 1090

France, Cedex 12

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Paris, Cedex 12, France, 75557

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Nantes, Cedex 1, France, 44093

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[Active, not recruiting]
Dijon, France, 21033

[Active, not recruiting]
Marseille, France, 13008

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Freiburg, Baden-Württemberg, Germany, 79106

[Active, not recruiting]
Heidelberg, Baden-Württemberg, Germany, 69120

[Active, not recruiting]
Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

[Active, not recruiting]
München, Bayern, Germany, 81675

[Active, not recruiting]
Regensburg, Bayern, Germany, 93053

Germany, Hessen

[Active, not recruiting]
Darmstadt, Hessen, Germany, 64297

[Active, not recruiting]
Frankfurt, Hessen, Germany, 60596

Germany, Niedersachsen

[Active, not recruiting]
Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

[Active, not recruiting]
Aachen, Nordrhein-Westfalen, Germany, 52074

[Active, not recruiting]
Bonn, Nordrhein-Westfalen, Germany, 53105

[Terminated]
Essen, Nordrhein-Westfalen, Germany, 45122

[Active, not recruiting]
Köln, Nordrhein-Westfalen, Germany, 50924

[Terminated]
Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

[Active, not recruiting]
Ludwigshafen, Rheinland-Pfalz, Germany, 67063

[Active, not recruiting]
Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

[Completed]
Homburg, Saarland, Germany, 66424

Germany, Sachsen

[Active, not recruiting]
Chemnitz, Sachsen, Germany, 09116

[Terminated]
Dresden, Sachsen, Germany, 01307

[Active, not recruiting]
Dresden, Sachsen, Germany, 06067

[Active, not recruiting]
Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

[Active, not recruiting]
Kiel, Schleswig-Holstein, Germany, 24105

[Active, not recruiting]
Lübeck, Schleswig-Holstein, Germany, 23538

Germany

[Terminated]
Berlin, Germany, 13353

[Active, not recruiting]
Hamburg, Germany, 20251

[Active, not recruiting]
Marburg, Germany, 35037

Hungary

[Active, not recruiting]
Budapest, Hungary, 1036

[Active, not recruiting]
Budapest, Hungary, 1089

[Active, not recruiting]
Budapest, Hungary, 1106

[Active, not recruiting]
Debrecen, Hungary, 4032

[Terminated]
Veszprem, Hungary, 8200

[Active, not recruiting]
Zalaegerszeg, Hungary, H-8900

Italy

[Active, not recruiting]
Ancona, Italy, 60126

[Active, not recruiting]
Bari, Italy, 70124

[Terminated]
Catania, Italy, 95123

[Active, not recruiting]
Firenze, Italy, 50134

[Active, not recruiting]
Milano, Italy, 20122

[Active, not recruiting]
Milano, Italy, 20132

[Terminated]
Milano, Italy, 20157

[Terminated]
Padova, Italy, 35128

[Active, not recruiting]
Roma, Italy, 00133

[Active, not recruiting]
Roma, Italy, 00198

[Terminated]
Torino, Italy, 10122

Japan, Aichi

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Nagoya, Aichi, Japan, 466-8560

[Active, not recruiting]
Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

[Active, not recruiting]
Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

[Active, not recruiting]
Suita, Osaka, Japan, 565-0871

Japan, Tokyo

[Active, not recruiting]
Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

[Active, not recruiting]
Kyoto, Japan, 606-8507

Korea, Republic of

[Active, not recruiting]
Incheon, Korea, Republic of, 405-760

[Active, not recruiting]
Kungki-do, Korea, Republic of, 463-707

[Active, not recruiting]
Seoul, Korea, Republic of, 110 744

[Active, not recruiting]
Seoul, Korea, Republic of, 110-744

[Active, not recruiting]
Seoul, Korea, Republic of, 138-736

[Recruiting]
Seoul, Korea, Republic of

Latvia

[Active, not recruiting]
Riga, Latvia, 1009

[Active, not recruiting]
Riga, Latvia, LV-1002

Singapore

[Active, not recruiting]
Singapore, Singapore, 119074

[Active, not recruiting]
Singapore, Singapore, 168751

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History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
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- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
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- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

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1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

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Study NCT01012973
on Date: August 26, 2010 (v8)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	August 2010
Overall Status:	Active, not recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	August 26, 2010

Last Update Posted:	August 27, 2010 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5.

Change in the NEI-VFQ-25 total score from baseline
Week 24

6. Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT)
- Adults >= 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1

- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations:

Australia, New South Wales

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Chemnitz, Sachsen, Germany, 09116

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Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

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Lübeck, Schleswig-Holstein, Germany, 23538

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Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

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Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1009

Riga, Latvia, LV-1002

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

IPDSharing

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ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

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7	○	○	August 25, 2010	Study Status and Contacts/Locations
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22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: September 8, 2010 (v9)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	September 2010
Overall Status:	Active, not recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	September 8, 2010

Last Update Posted:	September 9, 2010 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5.

Change in the NEI-VFQ-25 total score from baseline
Week 24

6. Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT)
- Adults >= 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1

- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
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Locations: **Australia, New South Wales**

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Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

Japan, Aichi

Nagoya, Aichi, Japan, 466-8560

Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

Suita, Osaka, Japan, 565-0871

Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1009

Riga, Latvia, LV-1002

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

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History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
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- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

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1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
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14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
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22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

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Study NCT01012973
on Date: September 19, 2011 (v15)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	September 2011
Overall Status:	Active, not recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Actual]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	September 19, 2011

Last Update Posted:	September 20, 2011 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	177 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Biological: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5.

Change in the NEI-VFQ-25 total score from baseline
Week 24

6. Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness ≥ 250 µm on optical coherence tomography (OCT)
- Adults ≥ 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1

- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations:

Australia, New South Wales

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66421

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Marburg, Germany, 35037

Hungary

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Budapest, Hungary, 1133

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

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Catania, Italy, 95123

Firenze, Italy, 50134

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Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

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Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

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History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

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Compare | Comparison Format: (•) Merged ( ) Side-by-Side

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Study NCT01012973
on Date: October 4, 2010 (v10)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	October 2010
Overall Status:	Active, not recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	October 4, 2010

Last Update Posted:	October 5, 2010 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
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   Week 24

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2. Change from baseline in BCVA score
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Change in the NEI-VFQ-25 total score from baseline
Week 24

6. Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT)
- Adults >= 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
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- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations:

Australia, New South Wales

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66424

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Marburg, Germany, 35037

Hungary

Budapest, Hungary, 1036

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

Japan, Aichi

Nagoya, Aichi, Japan, 466-8560

Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

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Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1009

Riga, Latvia, LV-1002

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

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History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

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5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
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Compare | Comparison Format: (•) Merged ( ) Side-by-Side

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Study NCT01012973
on Date: October 23, 2012 (v19)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	2009-010973-19 [EudraCT Number]

Study Status

Record Verification:	October 2012
Overall Status:	Completed
Study Start:	October 2009
Primary Completion:	February 2011 [Actual]
Study Completion:	February 2012 [Actual]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Results First Submitted:	October 23, 2012
	October 23, 2012

Results First Submitted that Met QC Criteria:	
Results First Posted:	November 22, 2012 [Estimate]
Certification/Extension First Submitted:	January 26, 2012
Certification/Extension First Submitted that Met QC Criteria:	January 26, 2012
Certification/Extension First Posted:	January 30, 2012 [Estimate]
Last Update Submitted that Met QC Criteria:	October 23, 2012
Last Update Posted:	November 22, 2012 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	

Macular Edema
Central Retinal Vein Occlusion
CRVO
VEGF Trap-Eye
best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	177 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.	Biological: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Intravitreal injection. Weeks 0 to 20 of Aflibercept Injection every 4 weeks; Weeks 24 to 52 every 4 weeks PRN (pro re nata, on demand); plus additional on Week 60 and 68.
Sham Comparator: Sham treatment Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept	Sham treatment Sham treatment. Weeks 0 to 52 sham treatment every 4 weeks; plus additional on Week 60 and 68.

Arms	Assigned Interventions
Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.	

Outcome Measures

[See Results Section.]

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness ≥ 250 µm on optical coherence tomography (OCT)
- Adults ≥ 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis

- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

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France, Cedex 1

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Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Marburg, Hessen, Germany, 35037

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Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

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Dresden, Sachsen, Germany, 01307

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Lübeck, Schleswig-Holstein, Germany, 23538

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Budapest, Hungary, 1106

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Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

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Roma, Italy, 00198

Torino, Italy, 10122

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Nagoya, Aichi, Japan, 467-8602

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Japan, Osaka

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Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

IPDSharing

Plan to Share IPD:

References

Citations:

Links:

Available IPD/Information:

Study Results

Participant Flow

Reporting Groups

	Description
Aflibercept Injection First, Then Aflibercept Injection	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment First, Then Aflibercept Injection	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Overall Study

	Aflibercept Injection First, Then Aflibercept Injection	Sham Treatment First, Then Aflibercept Injection
Started	106	71
Participants Received Treatment	104 [1]	68 [1]
Fulfilled Requirements of FAS Population	103 [2]	68 [2]
Completed Week 24, From FAS	97	57
Completed Week 52, From FAS	91	52
Completed	90	52
Not Completed	16	19
Adverse Event	5	5
Lack of Efficacy	0	5
Lost to Follow-up	1	0

	Aflibercept Injection First, Then Aflibercept Injection	Sham Treatment First, Then Aflibercept Injection
(Overseas travel - indefinite period)		
Increase in vis. acuity, never injected	0	1
Protocol Violation	5	2
Withdrawal by Subject	4	6

[1] Safety Population: Participants received treatment

[2] Full Analysis Set (FAS) Population: Participants received treatment with post baseline measurements

Baseline Characteristics

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Baseline Measures

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Overall Number of Participants		104	68	172
Age Continuous Mean (Standard Deviation) Unit of measure: Years	Number Analyzed	104 Participants	68 Participants	172 Participants
		60.0 (12.3)	63.8 (13.3)	61.5 (12.8)
Sex: Female, Male Measure type: Count of Participants Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
	Female	45 43.27%	31 45.59%	76 44.19%
	Male	59 56.73%	37 54.41%	96 55.81%
Ethnicity (NIH/OMB) Measure type: Count of Participants Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
	Hispanic or Latino	4 3.85%	1 1.47%	5 2.91%
	Not Hispanic or Latino	100 96.15%	66 97.06%	166 96.51%
	Unknown or Not Reported	0 0%	1 1.47%	1 0.58%
Baseline Best Corrected Visual Acuity (BCVA) letter scores [1] Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		53.5 (15.7)	50.9 (15.4)	52.5 (15.6)
	[1]	Only participants with a ETDRS (Early Treatment Diabetic Retinopathy Study) Best Corrected Visual Acuity letter score of 73 to 25 (= Acuity of 20/40 to 20/320) in the study eye at 4 meters were included; a higher score represents better functioning.		
	Number Analyzed	104 Participants	68 Participants	172 Participants

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Number of participants with baseline retinal perfusion [1] Measure type: Number Unit of measure: Participants				
Perfused		90	54	144
Nonperfused		7	7	14
Indeterminate		7	7	14
	[1] Retinal perfusion defined as less than 10 disc areas of capillary nonperfusion using fluorescein angiography (FA)			
Baseline Retinal Thickness by Optical Coherence Tomography (OCT) Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		682.78 (233.36)	638.66 (224.69)	665.34 (230.33)
Baseline intraocular pressure Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		15.2 (2.8)	14.4 (2.7)	14.9 (2.8)
Number of participants with time since Central retinal vein occlusion (CRVO) diagnosis Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
>= 2 months		46	33	79
< 2 months		56	35	91
Missing		2	0	2

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Baseline National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) total score [1]	Number Analyzed	104 Participants	68 Participants	172 Participants
		79.66 (13.06)	78.94 (14.00)	79.38 (13.40)

[1] The NEI VFQ-25 total score ranges from 0-100 with a score of 0 being the worst outcome and 100 being the best outcome. The NEI VFQ questionnaire is organized as a collection of subscales which are all scored from 0-100. To reach the overall composite score, each sub-scale score is averaged in order to give each sub-scale equal weight.

European questionnaire 5 dimensions (EQ-5D) total score [1] Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		0.87 (0.15)	0.86 (0.16)	0.87 (0.15)

[1] The EQ-5D total score ranges from -0.594 to 1.000 with -0.594 being the worst.

Race Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
Asian		26	15	41
White		75	49	124
Unknown or Not Reported		3	4	7

Outcome Measures

1. Primary Outcome Measure:

Measure Title	

	Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures
Measure Description	Defined study baseline range of Early Treatment Diabetic Retinopathy Study (ETDRS) Best Corrected Visual Acuity (BCVA) letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. Nominator = (Number of participants who maintained vision * 100); Denominator = Number of participants analyzed.
Time Frame	Baseline and Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures Measure Type: Number Unit of Measure: Percentage of participants	60.2	22.1

Statistical Analysis 1 for Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis of difference of Eylea minus Sham of 0 was tested. In the database close after Week 24, basis for primary efficacy evaluation, 56 Sham / 96 Eylea subjects were considered as week 24 completers.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	[Not specified]
	Method	Cochran-Mantel-Haenszel
	Comments	[Not specified]

	Estimation Parameter	CMH adjusted difference

Method of Estimation	Estimated Value	38.3
	Confidence Interval	(2-sided) 95% 24.4 to 52.1
	Estimation Comments	The estimate is calculated as Eylea minus Sham. A positive value shows Eylea showed a higher BCVA total score compared to Sham.

2. Secondary Outcome Measure:

Measure Title	Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF)
Measure Description	Defined study baseline range of ETDRS Best Corrected Visual Acuity letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. However, because this was assessed at the screening visit, subjects may have had a higher BCVA recorded at the baseline visit and would not have been excluded from the study.
Time Frame	Baseline and Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI

	depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF) Measure Type: Mean (Standard Deviation) Unit of Measure: Letters correctly read	71.6 (17.1)	54.3 (20.2)

Statistical Analysis 1 for Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF)

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis was equality in change from baseline to Week 24 in BCVA total letter score between Eylea and Sham. If primary efficacy was successful, secondary efficacy endpoints were tested in a pre-specified fixed sequence testing procedure. Change in BCVA letter

		score was to be tested first in this sequence.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	As primary efficacy evaluation was significant, and this p-value was below significance level of two-sided <.05, the fixed sequence testing did continue with next secondary endpoint.
	Method	ANOVA
	Comments	ANOVA, adjusting for region and baseline BCVA category as fixed factors.

Method of Estimation	Estimation Parameter	Difference in Least square means
	Estimated Value	14.7
	Confidence Interval	(2-sided) 95% 10.8 to 18.7
	Estimation Comments	The difference is calculated as Eylea minus Sham. A positive value indicates Eylea showed a higher change in BCVA total score until week 24 compared to Sham.

3. Secondary Outcome Measure:

Measure Title	Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF
Measure Description	
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	67
Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: microns	-448.58 (256.02)	-169.27 (224.72)

Statistical Analysis 1 for Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF

	Comparison Groups	

Statistical Analysis Overview		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis was equality in change from baseline to Week 24 in central retinal thickness between Eylea and Sham. If primary efficacy was successful, secondary efficacy end points were to be tested in a pre-specified fixed sequence testing procedure. Change in central retinal thickness was to be tested at second place in this sequence.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	As fixed sequence testing did reject nullhypothesis of change from baseline in BCVA until week 24, and this p-value was below significance level of two-sided <.05, the fixed sequence testing did continue with next secondary endpoint.
	Method	ANCOVA
	Comments	ANCOVA, stratified by region and baseline BCVA category, baseline central retinal thickness added as covariate.

Method of Estimation	Estimation Parameter	Difference in Least square (LS) means
	Estimated Value	-239.42
	Confidence Interval	(2-sided) 95% -286.31 to -192.53

	Estimation Comments	The difference is calculated as Eylea minus Sham. A negative value indicates Eylea showed a higher reduction in change in central retinal thickness until week 24 compared to Sham.

4. Secondary Outcome Measure:

Measure Title	Percentage of Participants Who Developed Neovascularization During the First 24 Weeks
Measure Description	Formation of blood vessels in the anterior segment, optic disc, or elsewhere in the fundus up to Week 24
Time Frame	From baseline until Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Percentage of Participants Who Developed Neovascularization During the First 24 Weeks Measure Type: Number Unit of Measure: Percentage of participants		
Any neovascularization	2.9	4.4
Anterior segment neovascularization	1.9	1.5
Neovascularization of the optic disc (NVD)	0.0	0.0
Neovascularization elsewhere in the fundus (NVE)	1.0	2.9

Statistical Analysis 1 for Percentage of Participants Who Developed Neovascularization During the First 24 Weeks

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Nullhypothesis of no difference in development of neovascularizations between Eylea and Sham group was tested. (Any neovascularization)
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	0.5947
	Comments	As fixed sequence testing did reject nullhypothesis of change from baseline in CRT until week 24, and this p-value was not below significance level of two-sided <.05,

		the fixed sequence testing did end with this evaluation.
	Method	Cochran-Mantel-Haenszel
	Comments	Cochrane-Mantel-Haenszel test, stratified by region and baseline BCVA category.

Method of Estimation	Estimation Parameter	CMH adjusted Difference
	Estimated Value	-1.5
	Confidence Interval	(2-sided) 95% -7.4 to 4.4
	Estimation Comments	[Not specified]

5. Secondary Outcome Measure:

Measure Title	Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF
Measure Description	The NEI VFQ-25 total score ranges from 0-100 with a score of 0 being the worst outcome and 100 being the best outcome. The NEI VFQ questionnaire is organized as a collection of subscales which are all scored from 0-100. To reach the overall composite score, each sub-scale score is averaged in order to give each sub-scale equal weight
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often

	as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	96	65
Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: Scores on a scale	7.46 (9.55)	3.55 (9.74)

Statistical Analysis 1 for Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	[Not specified]
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

	Estimation Parameter	Difference in LS means

Method of Estimation	Estimated Value	4.2
	Confidence Interval	(2-sided) 95% 1.7 to 6.8
	Estimation Comments	As the fixed sequence of secondary endpoints stopped with proportion of neovascularizations developed until week 24, 95% confidence interval is only of descriptive nature.

6. Secondary Outcome Measure:

Measure Title	Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF
Measure Description	EQ-5D is a quality of life questionnaire based on a scale from -0.594 (worst) to 1.00 (best).
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment,

	who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	95	64
Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: Scores on a scale	0.029 (0.139)	-0.002 (0.195)

Statistical Analysis 1 for Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	[Not specified]
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Method of Estimation	Estimation Parameter	Difference in LS Means
	Estimated Value	0.044
	Confidence Interval	(2-sided) 95% -0.002 to 0.09
	Estimation Comments	As the fixed sequence of secondary endpoints stopped with proportion of neovascularizations developed until week 24, 95% confidence interval is only of descriptive nature.

Reported Adverse Events

Time Frame	[Not specified]
Adverse Event Reporting Description	[Not specified]

Reporting Groups

	Description
Aflibercept Injection (Until Week 20)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20. Participants were observed until Week 24. Participants in the safety population were at risk.
Sham Treatment (Until Week 20)	Participants received sham treatment administered every 4 weeks from Day 1 through Week 20. Participants were observed until Week 24. Participants in the safety population were at risk.
Aflibercept Injection (Until Week 48)	Participants who continued the study drug until Week 24 received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Participants were observed from Week 24 until Week 52. Participants in the safety population that completed Week 24 were at risk.
Sham Treatment (Until Week 48)	Participants who continued the study drug until Week 24 received sham treatment administered every 4 weeks from Week 24 to Week 48. Participants were observed from Week 24 until Week 52. Participants in the safety population that completed Week 24 were at risk.
Aflibercept Injection Continued (Until Week 68)	Participants on IAI who continued the study drug until Week 52, received 2 mg dose of IAI depending on the study retreatment criteria at Week 52, 60 and 68. Participants were observed starting from Week 52. Participants in the safety population that completed Week 52 were at risk.

Sham Treatment Then Aflibercept Injection (Until Week 68)	Participants on sham treatment switched to IAI, received a 2 mg dose of IAI at Week 52 and depending on the study retreatment criteria at Week 60 and 68. Participants were observed starting from Week 52. Participants in the safety population that completed Week 52 were at risk.

All-Cause Mortality

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)
Total						

Serious Adverse Events

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)
Total	8/104 (7.69%)	8/68 (11.76%)	14/97 (14.43%)	7/57 (12.28%)	4/91 (4.4%)	3/52 (5.77%)
Cardiac disorders						
Aortic valve incompetence [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Cardiac failure [A*]						

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Coronary artery stenosis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Diastolic dysfunction [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Mitral valve incompetence [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Eye disorders						
Blindness unilateral [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Glaucoma [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Iris neovascularisation [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular fibrosis [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular ischaemia [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular oedema [A*]	0/104 (0%)	2/68 (2.94%)	4/97 (4.12%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Retinal vein occlusion [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Visual acuity reduced [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	0/57 (0%)	2/91 (2.2%)	0/52 (0%)
Vitreous detachment [A*]	1/104	0/68	0/97	0/57	0/91	0/52

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Vitreous haemorrhage [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Gastrointestinal disorders						
Diverticular perforation [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Hepatobiliary disorders						
Hepatic function abnormal [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Infections and infestations						
Furuncle [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Gastroenteritis [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Pneumonia [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Vestibular neuronitis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Injury, poisoning and procedural complications						
Fall [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Femur fracture [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Hand fracture [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Humerus fracture [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Radius fracture [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Spinal compression fracture [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Musculoskeletal and connective tissue disorders						
Intervertebral disc protrusion [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Spinal column stenosis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)						
Breast cancer [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Oropharyngeal cancer stage unspecified [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Nervous system disorders						
Paraesthesia [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Syncope [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	2/57 (3.51%)	0/91 (0%)	0/52 (0%)
Transient ischaemic attack [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Respiratory, thoracic and mediastinal disorders						
Dyspnoea [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Laryngeal granuloma [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Pulmonary hypertension [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Surgical and medical procedures						
Ischaemic heart disease prophylaxis [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vascular disorders						
Circulatory collapse [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)

* Indicates events were collected by non-systematic methods.

A Term from vocabulary, MedDRA (14.1)

Other Adverse Events

Frequency Threshold Above Which Other Adverse Events are Reported: 5%

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)
Total	52/104 (50%)	44/68 (64.71%	66/97 (68.04%	30/57 (52.63%	38/91 (41.76%	19/52 (36.54%
Blood and lymphatic system disorders						
Anaemia [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Eye disorders						
Conjunctival haemorrhage [A+]	10/104 (9.62%)	3/68 (4.41%)	3/97 (3.09%)	0/57 (0%)	9/91 (9.89%)	3/52 (5.77%)
Eye irritation [A+]	3/104 (2.88%)	7/68 (10.29%	4/97 (4.12%)	1/57 (1.75%)	1/91 (1.1%)	2/52 (3.85%)
Eye pain [A+]	12/104 (11.54%	3/68 (4.41%)	6/97 (6.19%)	2/57 (3.51%)	1/91 (1.1%)	0/52 (0%)
Foreign body sensation in eyes [A+]	6/104 (5.77%)	5/68 (7.35%)	2/97 (2.06%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Lacrimation increased [A+]	3/104 (2.88%)	4/68 (5.88%)	3/97 (3.09%)	4/57 (7.02%)	1/91 (1.1%)	2/52 (3.85%)
Macular fibrosis [A+]	1/104 (0.96%)	1/68 (1.47%)	5/97 (5.15%)	4/57 (7.02%)	0/91 (0%)	3/52 (5.77%)
Macular ischaemia [A+]	7/104 (6.73%)	5/68 (7.35%)	3/97 (3.09%)	1/57 (1.75%)	0/91 (0%)	1/52 (1.92%)
Macular oedema [A+]	2/104 (1.92%)	9/68 (13.24%	30/97 (30.93%	7/57 (12.28%	17/91 (18.68%	2/52 (3.85%)
Ocular hyperaemia [A+]	5/104 (4.81%)	4/68 (5.88%)	2/97 (2.06%)	1/57 (1.75%)	4/91 (4.4%)	1/52 (1.92%)
Optic disc vascular disorder [A+]	5/104 (4.81%)	3/68 (4.41%)	3/97 (3.09%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Retinal exudates [A+]	8/104 (7.69%)	5/68 (7.35%)	4/97 (4.12%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Retinal haemorrhage [A+]	4/104 (3.85%)	6/68 (8.82%)	11/97 (11.34%	5/57 (8.77%)	5/91 (5.49%)	2/52 (3.85%)
Retinal vascular disorder [A+]	6/104	7/68	10/97	2/57	0/91	2/52

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Visual acuity reduced [A]	2/104 (1.92%)	7/68 (10.29%)	10/97 (10.31%)	1/57 (1.75%)	7/91 (7.69%)	1/52 (1.92%)
Vitreous detachment [A]	2/104 (1.92%)	1/68 (1.47%)	7/97 (7.22%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vitreous floaters [A]	6/104 (5.77%)	0/68 (0%)	1/97 (1.03%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Gastrointestinal disorders						
Nausea [A]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Infections and infestations						
Influenza [A]	2/104 (1.92%)	0/68 (0%)	5/97 (5.15%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Nasopharyngitis [A]	8/104 (7.69%)	6/68 (8.82%)	10/97 (10.31%)	11/57 (19.3%)	4/91 (4.4%)	2/52 (3.85%)
Investigations						
Intraocular pressure increased [A]	9/104 (8.65%)	4/68 (5.88%)	14/97 (14.43%)	2/57 (3.51%)	2/91 (2.2%)	1/52 (1.92%)
Visual acuity tests abnormal [A]	0/104 (0%)	1/68 (1.47%)	5/97 (5.15%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Musculoskeletal and connective tissue disorders						
Arthralgia [A]	1/104 (0.96%)	5/68 (7.35%)	2/97 (2.06%)	1/57 (1.75%)	2/91 (2.2%)	0/52 (0%)
Nervous system disorders						
Headache [A]	7/104 (6.73%)	4/68 (5.88%)	4/97 (4.12%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Vascular disorders						
Hypertension [A*]	4/104 (3.85%)	3/68 (4.41%)	4/97 (4.12%)	4/57 (7.02%)	3/91 (3.3%)	2/52 (3.85%)

* Indicates events were collected by non-systematic methods.

A Term from vocabulary, MedDRA (14.1)

Limitations and Caveats

[Not specified]

More Information

Certain Agreements:

Principal Investigators are NOT employed by the organization sponsoring the study.

There IS an agreement between the Principal Investigator and the Sponsor (or its agents) that restricts the PI's rights to discuss or publish trial results after the trial is completed.

Publishing of result communication only after Bayer´s written approval. Manuscript to Bayer sixty days before public release. If no written Bayer comment within 60 days consider approval given. If multi-site study, principal investigator (PI) not do independently publish results before publication of the multi-site paper, but PI not restricted from 24 months from study to completion onwards.

Results Point of Contact:

Name/Official Title: Therapeutic Area Head
Organization: BAYER
Phone:
Email: clinical-trials-contact@bayerhealthcare.com

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U.S. National Library of Medicine
ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
- Additions will be displayed in *green*.
- The yellow choices in the table indicate the study versions currently compared below. A yellow row indicates the study version being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: October 27, 2014 (v23)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	2009-010973-19 [EudraCT Number]

Study Status

Record Verification:	October 2014
Overall Status:	Completed
Study Start:	October 2009
Primary Completion:	February 2011 [Actual]
Study Completion:	February 2012 [Actual]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Results First Submitted:	October 23, 2012
	October 23, 2012

Results First Submitted that Met QC Criteria:	
Results First Posted:	November 22, 2012 [Estimate]
Certification/Extension First Submitted:	January 26, 2012
Certification/Extension First Submitted that Met QC Criteria:	January 26, 2012
Certification/Extension First Posted:	January 30, 2012 [Estimate]
Last Update Submitted that Met QC Criteria:	October 27, 2014
Last Update Posted:	November 3, 2014 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	Sponsor
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion

CRVO
VEGF Trap-Eye
best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	177 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.	Biological: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Intravitreal injection. Weeks 0 to 20 of Aflibercept Injection every 4 weeks; Weeks 24 to 52 every 4 weeks PRN (pro re nata, on demand); plus additional on Week 60 and 68. Sham treatment Sham treatment. Weeks 0 to 52 sham treatment every 4 weeks; plus additional on Week 60 and 68.
Sham Comparator: Sham treatment Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the	Sham treatment Sham treatment. Weeks 0 to 52 sham treatment every 4 weeks; plus additional on Week 60 and 68.

Arms	Assigned Interventions
study retreatment criteria at Week 60 and 68.	

Outcome Measures

[See Results Section]

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness ≥ 250 µm on optical coherence tomography (OCT)
- Adults ≥ 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis

- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Marburg, Hessen, Germany, 35037

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66421

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Hungary

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Budapest, Hungary, 1133

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

Japan, Aichi

Nagoya, Aichi, Japan, 466-8560

Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

Suita, Osaka, Japan, 565-0871

Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of, Gyeonggido

Seongnam-si, Gyeonggido, Korea, Republic of, 463-707

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 137-701

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

IPDSharing

Plan to Share IPD:

References

Citations: [Study Results] Holz FG, Roider J, Ogura Y, Korobelnik JF, Simader C, Groetzbach G, Vitti R, Berliner AJ, Hiemeyer F, Beckmann K, Zeitz O, Sandbrink R. VEGF Trap-Eye for macular oedema secondary to central retinal vein occlusion: 6-month results of the phase III GALILEO study. Br J Ophthalmol. 2013 Mar;97(3):278-84. doi: 10.1136/bjophthalmol-2012-301504. Epub 2013 Jan 7.

Erratum in: Br J Ophthalmol. 2015 Dec;99(12):1746. PubMed 23298885

[Study Results] Korobelnik JF, Holz FG, Roider J, Ogura Y, Simader C, Schmidt-Erfurth U, Lorenz K, Honda M, Vitti R, Berliner AJ, Hiemeyer F, Stemper B, Zeitz O, Sandbrink R; GALILEO Study Group. Intravitreal Aflibercept Injection for Macular Edema Resulting from Central Retinal Vein Occlusion: One-Year Results of the Phase 3 GALILEO Study. Ophthalmology. 2014 Jan;121(1):202-208. doi: 10.1016/j.ophtha.2013.08.012. Epub 2013 Sep 29. PubMed 24084497

Links:

Available IPD/Information:

Study Results

Participant Flow

Reporting Groups

	Description
Aflibercept Injection First, Then Aflibercept Injection	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment First, Then Aflibercept Injection	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Overall Study

	Aflibercept Injection First, Then Aflibercept Injection	Sham Treatment First, Then Aflibercept Injection
Started	106	71
Participants Received Treatment	104 [1]	68 [1]
Fulfilled Requirements of FAS Population	103 [2]	68 [2]
Completed Week 24, From FAS	97	57
Completed Week 52, From FAS	91	52
Completed	90	52
Not Completed	16	19
Adverse Event	5	5
Lack of Efficacy	0	5
Lost to Follow-up	1	0
(Overseas travel - indefinite period)	1	0
Increase in vis. acuity, never injected	0	1
Protocol Violation	5	2
Withdrawal by Subject	4	6

[1] Safety Population: Participants received treatment

[2] Full Analysis Set (FAS) Population: Participants received treatment with post baseline measurements

Baseline Characteristics

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every

	4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Baseline Measures

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Overall Number of Participants		104	68	172
Age, Continuous Mean (Standard Deviation) Unit of measure: Years	Number Analyzed	104 Participants	68 Participants	172 Participants
		60.0 (12.3)	63.8 (13.3)	61.5 (12.8)
Sex: Female, Male Measure type: Count of Participants Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
	Female	45 43.27%	31 45.59%	76 44.19%
	Male	59 56.73%	37 54.41%	96 55.81%
Ethnicity (NIH/OMB) Measure type: Count of Participants Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
	Hispanic or Latino	4 3.85%	1 1.47%	5 2.91%
	Not Hispanic or Latino	100 96.15%	66 97.06%	166 96.51%
		0 0%	1 1.47%	1 0.58%

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
	Unknown or Not Reported			
Baseline Best Corrected Visual Acuity (BCVA) letter scores [1] Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		53.5 (15.7)	50.9 (15.4)	52.5 (15.6)
	[1]	Information retrieved from all baseline participants. Only participants with a ETDRS (Early Treatment Diabetic Retinopathy Study) Best Corrected Visual Acuity letter score of 73 to 25 (= Acuity of 20/40 to 20/320) in the study eye at 4 meters were included; a higher score represents better functioning.		
Number of participants with baseline retinal perfusion [1] Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
Perfused		90	54	144
Nonperfused		7	7	14
Indeterminate		7	7	14
	[1]	Retinal perfusion defined as less than 10 disc areas of capillary nonperfusion using fluorescein angiography (FA)		
Baseline Retinal Thickness by Optical Coherence Tomography (OCT) Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		682.78 (233.36)	638.66 (224.69)	665.34 (230.33)

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Baseline intraocular pressure Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		15.2 (2.8)	14.4 (2.7)	14.9 (2.8)
Number of participants with time since Central retinal vein occlusion (CRVO) diagnosis Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
>= 2 months		46	33	79
< 2 months		56	35	91
Missing		2	0	2
Baseline National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) total score [1]	Number Analyzed	104 Participants	68 Participants	172 Participants
		79.66 (13.06)	78.94 (14.00)	79.38 (13.40)
	[1]	The NEI VFQ-25 total score ranges from 0-100 with a score of 0 being the worst outcome and 100 being the best outcome. The NEI VFQ questionnaire is organized as a collection of subscales which are all scored from 0-100. To reach the overall composite score, each sub-scale score is averaged in order to give each sub-scale equal weight.		
European questionnaire 5 dimensions (EQ-5D) total score [1] Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		0.87 (0.15)	0.86 (0.16)	0.87 (0.15)

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Unit of measure: score on a scale				
	[1] The EQ-5D total score ranges from -0.594 to 1.000 with -0.594 being the worst.			
Race Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
Asian		26	15	41
White		75	49	124
Unknown or Not Reported		3	4	7

Outcome Measures

1. Primary Outcome Measure:

Measure Title	Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures
Measure Description	Defined study baseline range of Early Treatment Diabetic Retinopathy Study (ETDRS) Best Corrected Visual Acuity (BCVA) letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. Nominator = (Number of participants who maintained vision * 100); Denominator = Number of participants analyzed.
Time Frame	Baseline and Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures Measure Type: Number Unit of Measure: Percentage of participants	60.2	22.1

Statistical Analysis 1 for Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis of difference of Eylea minus Sham of 0 was tested. In the database close after Week 24, basis for primary efficacy evaluation, 56 Sham / 96 Eylea subjects were considered as week 24 completers.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	[Not specified]
	Method	Cochran-Mantel-Haenszel
	Comments	[Not specified]

Method of Estimation	Estimation Parameter	CMH adjusted difference
	Estimated Value	38.3
	Confidence Interval	(2-sided) 95% 24.4 to 52.1
	Estimation Comments	The estimate is calculated as Eylea minus Sham. A positive value shows Eylea showed a higher BCVA total score compared to Sham.

2. Secondary Outcome Measure:

Measure Title	Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF)
Measure Description	Defined study baseline range of ETDRS Best Corrected Visual Acuity letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. However, because this was assessed at the screening visit,

	subjects may have had a higher BCVA recorded at the baseline visit and would not have been excluded from the study.
Time Frame	Baseline and Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF) Measure Type: Mean (Standard Deviation) Unit of Measure: Letters correctly read	71.6 (17.1)	54.3 (20.2)

Statistical Analysis 1 for Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF)

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis was equality in change from baseline to Week 24 in BCVA total letter score between Eylea and Sham. If primary efficacy was successful, secondary efficacy endpoints were tested in a pre-specified fixed sequence testing procedure. Change in BCVA letter score was to be tested first in this sequence.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	As primary efficacy evaluation was significant, and this p-value was below significance level of two-sided <.05, the fixed sequence testing did continue with next secondary endpoint.
	Method	ANOVA
	Comments	ANOVA, adjusting for region and baseline BCVA category as fixed factors.

Method of Estimation	Estimation Parameter	Difference in Least square means
	Estimated Value	14.7
	Confidence Interval	(2-sided) 95% 10.8 to 18.7
	Estimation Comments	The difference is calculated as Eylea minus Sham. A positive value

		indicates Eylea showed a higher change in BCVA total score until week 24 compared to Sham.

3. Secondary Outcome Measure:

Measure Title	Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF
Measure Description	
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	67

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: microns	-448.58 (256.02)	-169.27 (224.72)

Statistical Analysis 1 for Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis was equality in change from baseline to Week 24 in central retinal thickness between Eylea and Sham. If primary efficacy was successful, secondary efficacy end points were to be tested in a pre-specified fixed sequence testing procedure. Change in central retinal thickness was to be tested at second place in this sequence.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	As fixed sequence testing did reject nullhypothesis of change from baseline in BCVA until week 24, and this p-value was below significance level of two-sided <.05, the fixed sequence testing did continue with next secondary endpoint.
	Method	ANCOVA
	Comments	ANCOVA, stratified by region and baseline BCVA category, baseline

		central retinal thickness added as covariate.

Method of Estimation	Estimation Parameter	Difference in Least square (LS) means
	Estimated Value	-239.42
	Confidence Interval	(2-sided) 95% -286.31 to -192.53
	Estimation Comments	The difference is calculated as Eylea minus Sham. A negative value indicates Eylea showed a higher reduction in change in central retinal thickness until week 24 compared to Sham.

4. Secondary Outcome Measure:

Measure Title	Percentage of Participants Who Developed Neovascularization During the First 24 Weeks
Measure Description	Formation of blood vessels in the anterior segment, optic disc, or elsewhere in the fundus up to Week 24
Time Frame	From baseline until Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-

	up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Percentage of Participants Who Developed Neovascularization During the First 24 Weeks Measure Type: Number Unit of Measure: Percentage of participants		
Any neovascularization	2.9	4.4
Anterior segment neovascularization	1.9	1.5
Neovascularization of the optic disc (NVD)	0.0	0.0
Neovascularization elsewhere in the fundus (NVE)	1.0	2.9

Statistical Analysis 1 for Percentage of Participants Who Developed Neovascularization During the First 24 Weeks

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Nullhypothesis of no difference in development of neovascularizations between Eylea and Sham group was tested. (Any neovascularization)
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	0.5947
	Comments	

		As fixed sequence testing did reject nullhypothesis of change from baseline in CRT until week 24, and this p-value was not below significance level of two-sided <.05, the fixed sequence testing did end with this evaluation.
	Method	Cochran-Mantel-Haenszel
	Comments	Cochrane-Mantel-Haenszel test, stratified by region and baseline BCVA category.

Method of Estimation	Estimation Parameter	CMH adjusted Difference
	Estimated Value	-1.5
	Confidence Interval	(2-sided) 95% -7.4 to 4.4
	Estimation Comments	[Not specified]

5. Secondary Outcome Measure:

Measure Title	Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF
Measure Description	The NEI VFQ-25 total score ranges from 0-100 with a score of 0 being the worst outcome and 100 being the best outcome. The NEI VFQ questionnaire is organized as a collection of subscales which are all scored from 0-100. To reach the overall composite score, each sub-scale score is averaged in order to give each sub-scale equal weight
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	96	65
Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: Scores on a scale	7.46 (9.55)	3.55 (9.74)

Statistical Analysis 1 for Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	[Not specified]
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Method of Estimation	Estimation Parameter	Difference in LS means
	Estimated Value	4.2
	Confidence Interval	(2-sided) 95% 1.7 to 6.8
	Estimation Comments	As the fixed sequence of secondary endpoints stopped with proportion of neovascularizations developed until week 24, 95% confidence interval is only of descriptive nature.

6. Secondary Outcome Measure:

Measure Title	Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF
Measure Description	EQ-5D is a quality of life questionnaire based on a scale from -0.594 (worst) to 1.00 (best).
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and

	depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	95	64
Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: Scores on a scale	0.029 (0.139)	-0.002 (0.195)

Statistical Analysis 1 for Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	[Not specified]
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Method of Estimation	Estimation Parameter	Difference in LS Means
	Estimated Value	0.044
	Confidence Interval	(2-sided) 95% -0.002 to 0.09
	Estimation Comments	As the fixed sequence of secondary endpoints stopped with proportion of neovascularizations developed until week 24, 95% confidence interval is only of descriptive nature.

Reported Adverse Events

Time Frame	[Not specified]
	[Not specified]

Adverse Event Reporting Description	

Reporting Groups

	Description
Aflibercept Injection (Until Week 20)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20. Participants were observed until Week 24. Participants in the safety population were at risk.
Sham Treatment (Until Week 20)	Participants received sham treatment administered every 4 weeks from Day 1 through Week 20. Participants were observed until Week 24. Participants in the safety population were at risk.
Aflibercept Injection (Until Week 48)	Participants who continued the study drug until Week 24 received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Participants were observed from Week 24 until Week 52. Participants in the safety population that completed Week 24 were at risk.
Sham Treatment (Until Week 48)	Participants who continued the study drug until Week 24 received sham treatment administered every 4 weeks from Week 24 to Week 48. Participants were observed from Week 24 until Week 52. Participants in the safety population that completed Week 24 were at risk.
Aflibercept Injection Continued (Until Week 68)	Participants on IAI who continued the study drug until Week 52, received 2 mg dose of IAI depending on the study retreatment criteria at Week 52, 60 and 68. Participants were observed starting from Week 52. Participants in the safety population that completed Week 52 were at risk.
Sham Treatment Then Aflibercept Injection (Until Week 68)	Participants on sham treatment switched to IAI, received a 2 mg dose of IAI at Week 52 and depending on the study retreatment criteria at Week

	60 and 68. Participants were observed starting from Week 52. Participants in the safety population that completed Week 52 were at risk.

All-Cause Mortality

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)
Total	/	/	/	/	/	/

Serious Adverse Events

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)	Affected/A Risk (%)
Total	8/104 (7.69%)	8/68 (11.76%	14/97 (14.43%	7/57 (12.28%	4/91 (4.4%)	3/52 (5.77%)
Cardiac disorders						
Aortic valve incompetence [A-]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Cardiac failure [A-]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Coronary artery stenosis [A-]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Diastolic dysfunction [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Mitral valve incompetence [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Eye disorders						
Blindness unilateral [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Glaucoma [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Iris neovascularisation [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular fibrosis [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular ischaemia [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular oedema [A*]	0/104 (0%)	2/68 (2.94%)	4/97 (4.12%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Retinal vein occlusion [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Visual acuity reduced [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	0/57 (0%)	2/91 (2.2%)	0/52 (0%)
Vitreous detachment [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vitreous haemorrhage [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Gastrointestinal disorders						

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Diverticular perforation [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Hepatobiliary disorders						
Hepatic function abnormal [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Infections and infestations						
Furuncle [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Gastroenteritis [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Pneumonia [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Vestibular neuronitis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Injury, poisoning and procedural complications						
Fall [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Femur fracture [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Hand fracture [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Humerus fracture [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Radius fracture [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Spinal compression fracture [A*]						

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Musculoskeletal and connective tissue disorders						
Intervertebral disc protrusion [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Spinal column stenosis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)						
Breast cancer [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Oropharyngeal cancer stage unspecified [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Nervous system disorders						
Paraesthesia [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Syncope [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	2/57 (3.51%)	0/91 (0%)	0/52 (0%)
Transient ischaemic attack [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Respiratory, thoracic and mediastinal disorders						
Dyspnoea [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Laryngeal granuloma [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Pulmonary hypertension [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Surgical and medical procedures						
Ischaemic heart disease prophylaxis [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vascular disorders						
Circulatory collapse [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)

* Indicates events were collected by non-systematic methods.

A Term from vocabulary, MedDRA (14.1)

Other Adverse Events

Frequency Threshold Above Which Other Adverse Events are Reported: 5%

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)
Total	52/104 (50%)	44/68 (64.71%)	66/97 (68.04%)	30/57 (52.63%)	38/91 (41.76%)	19/52 (36.54%)
Blood and lymphatic system disorders						
Anaemia [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Eye disorders						
Conjunctival haemorrhage [A*]						

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
	10/104 (9.62%)	3/68 (4.41%)	3/97 (3.09%)	0/57 (0%)	9/91 (9.89%)	3/52 (5.77%)
Eye irritation [A*]	3/104 (2.88%)	7/68 (10.29%	4/97 (4.12%)	1/57 (1.75%)	1/91 (1.1%)	2/52 (3.85%)
Eye pain [A*]	12/104 (11.54%	3/68 (4.41%)	6/97 (6.19%)	2/57 (3.51%)	1/91 (1.1%)	0/52 (0%)
Foreign body sensation in eyes [A*]	6/104 (5.77%)	5/68 (7.35%)	2/97 (2.06%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Lacrimation increased [A*]	3/104 (2.88%)	4/68 (5.88%)	3/97 (3.09%)	4/57 (7.02%)	1/91 (1.1%)	2/52 (3.85%)
Macular fibrosis [A*]	1/104 (0.96%)	1/68 (1.47%)	5/97 (5.15%)	4/57 (7.02%)	0/91 (0%)	3/52 (5.77%)
Macular ischaemia [A*]	7/104 (6.73%)	5/68 (7.35%)	3/97 (3.09%)	1/57 (1.75%)	0/91 (0%)	1/52 (1.92%)
Macular oedema [A*]	2/104 (1.92%)	9/68 (13.24%	30/97 (30.93%	7/57 (12.28%	17/91 (18.68%	2/52 (3.85%)
Ocular hyperaemia [A*]	5/104 (4.81%)	4/68 (5.88%)	2/97 (2.06%)	1/57 (1.75%)	4/91 (4.4%)	1/52 (1.92%)
Optic disc vascular disorder [A*]	5/104 (4.81%)	3/68 (4.41%)	3/97 (3.09%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Retinal exudates [A*]	8/104 (7.69%)	5/68 (7.35%)	4/97 (4.12%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Retinal haemorrhage [A*]	4/104 (3.85%)	6/68 (8.82%)	11/97 (11.34%	5/57 (8.77%)	5/91 (5.49%)	2/52 (3.85%)
Retinal vascular disorder [A*]	6/104 (5.77%)	7/68 (10.29%	10/97 (10.31%	2/57 (3.51%)	0/91 (0%)	2/52 (3.85%)

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
	2/104 (1.92%)	7/68 (10.29%)	10/97 (10.31%)	1/57 (1.75%)	7/91 (7.69%)	1/52 (1.92%)
Vitreous detachment [A*]	2/104 (1.92%)	1/68 (1.47%)	7/97 (7.22%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vitreous floaters [A*]	6/104 (5.77%)	0/68 (0%)	1/97 (1.03%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Gastrointestinal disorders						
Nausea [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Infections and infestations						
Influenza [A*]	2/104 (1.92%)	0/68 (0%)	5/97 (5.15%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Nasopharyngitis [A*]	8/104 (7.69%)	6/68 (8.82%)	10/97 (10.31%)	11/57 (19.3%)	4/91 (4.4%)	2/52 (3.85%)
Investigations						
Intraocular pressure increased [A*]	9/104 (8.65%)	4/68 (5.88%)	14/97 (14.43%)	2/57 (3.51%)	2/91 (2.2%)	1/52 (1.92%)
Visual acuity tests abnormal [A*]	0/104 (0%)	1/68 (1.47%)	5/97 (5.15%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Musculoskeletal and connective tissue disorders						
Arthralgia [A*]	1/104 (0.96%)	5/68 (7.35%)	2/97 (2.06%)	1/57 (1.75%)	2/91 (2.2%)	0/52 (0%)
Nervous system disorders						
Headache [A*]	7/104 (6.73%)	4/68 (5.88%)	4/97 (4.12%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Vascular disorders						

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Hypertension [A]*	4/104 (3.85%)	3/68 (4.41%)	4/97 (4.12%)	4/57 (7.02%)	3/91 (3.3%)	2/52 (3.85%)

* Indicates events were collected by non-systematic methods.

A Term from vocabulary, MedDRA (14.1)

Limitations and Caveats

[Not specified]

More Information

Certain Agreements:

Principal Investigators are NOT employed by the organization sponsoring the study.

There IS an agreement between the Principal Investigator and the Sponsor (or its agents) that restricts the PI's rights to discuss or publish trial results after the trial is completed.

Publishing of result communication only after Bayer´s written approval. Manuscript to Bayer sixty days before public release. If no written Bayer comment within 60 days consider approval given. If multi-site study, principal investigator (PI) not do independently publish results before publication of the multi-site paper, but PI not restricted from 24 months from study to completion onwards.

Results Point of Contact:

Name/Official Title: Therapeutic Area Head
Organization: BAYER
Phone:
Email: clinical-trials-contact@bayerhealthcare.com

Scroll up to access the controls Scroll to the Study top

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ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
- Additions will be displayed in *green*.
- The yellow choices in the table indicate the study versions currently compared below. A yellow row indicates the study version being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: November 1, 2010 (v11)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	November 2010
Overall Status:	Active, not recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	November 1, 2010

Last Update Posted:	November 2, 2010 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5.

Change in the NEI-VFQ-25 total score from baseline
Week 24

6. Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT)
- Adults >= 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1

- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

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München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

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Ludwigshafen, Rheinland-Pfalz, Germany, 67063

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Germany, Saarland

Homburg, Saarland, Germany, 66424

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Marburg, Germany, 35037

Hungary

Budapest, Hungary, 1036

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

Japan, Aichi

Nagoya, Aichi, Japan, 466-8560

Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

Suita, Osaka, Japan, 565-0871

Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1009

Riga, Latvia, LV-1002

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

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ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
- Additions will be displayed in *green*.
- The yellow choices in the table indicate the study versions currently compared below. A yellow row indicates the study version being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: November 12, 2009 (v1)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19 GALILEO

Study Status

Record Verification:	November 2009
Overall Status:	Recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	August 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
	November 12, 2009

Last Update Submitted that Met QC Criteria:	
Last Update Posted:	November 13, 2009 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion.
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor

Allocation: Randomized

Enrollment: 165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; weeks 52 to 100 safety follow-up.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; weeks 52 to 100 safety follow-up.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5. Change in the NEI-VFQ-25 total score from baseline
   Week 24
6.

Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT).
- Adults >= 18 years.
- early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye.

Exclusion Criteria:

- Previous treatment with anti-angiogenic drugs in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.)
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Central Contact: Bayer Clinical Trials Contact
Email: clinical-trials-contact@bayerhealthcare.com

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

[Not yet recruiting]
Chatswood, New South Wales, Australia, 2067

[Not yet recruiting]
Sydney, New South Wales, Australia, 2000

[Not yet recruiting]
Sydney, New South Wales, Australia, 2145

Australia, Victoria

[Not yet recruiting]
East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

[Not yet recruiting]
Nedlands, Western Australia, Australia, 6009

Australia

[Not yet recruiting]
Parramatta, Australia, 2150

Austria, Oberösterreich

[Not yet recruiting]
Linz, Oberösterreich, Austria, 4020

Austria, Tirol

[Not yet recruiting]
Innsbruck, Tirol, Austria, 6020

Austria

[Not yet recruiting]
Linz, Austria, 4021

[Not yet recruiting]
Wien, Austria, 1090

France, Cedex 12

[Not yet recruiting]
Paris, Cedex 12, France, 75557

France

[Not yet recruiting]
Bordeaux, France, 33000

[Not yet recruiting]
Dijon Cedex, France, BP 1542-21

[Not yet recruiting]
Marseille, France, 13008

[Not yet recruiting]
Nantes Cedex, France, 44035

[Not yet recruiting]
Paris, France, 75015

Germany, Baden-Württemberg

[Not yet recruiting]
Freiburg, Baden-Württemberg, Germany, 79106

[Not yet recruiting]
Heidelberg, Baden-Württemberg, Germany, 69120

[Not yet recruiting]
Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

[Not yet recruiting]
München, Bayern, Germany, 81675

[Not yet recruiting]
Regensburg, Bayern, Germany, 93053

Germany, Hessen

[Recruiting]
Darmstadt, Hessen, Germany, 64276

[Not yet recruiting]
Marburg, Hessen, Germany, 35043

Germany, Niedersachsen

[Not yet recruiting]
Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

[Not yet recruiting]
Aachen, Nordrhein-Westfalen, Germany, 52074

[Not yet recruiting]
Bonn, Nordrhein-Westfalen, Germany, 53105

[Not yet recruiting]
Essen, Nordrhein-Westfalen, Germany, 45147

[Not yet recruiting]
Köln, Nordrhein-Westfalen, Germany, 50931

Germany, Rheinland-Pfalz

[Not yet recruiting]
Ludwigshafen, Rheinland-Pfalz, Germany, 67063

[Not yet recruiting]
Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

[Not yet recruiting]
Homburg, Saarland, Germany, 66421

Germany, Sachsen

[Not yet recruiting]
Chemnitz, Sachsen, Germany, 09116

[Not yet recruiting]
Dresden, Sachsen, Germany, 01067

[Not yet recruiting]
Dresden, Sachsen, Germany, 01307

[Not yet recruiting]
Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

[Not yet recruiting]
Kiel, Schleswig-Holstein, Germany, 24105

[Not yet recruiting]
Lübeck, Schleswig-Holstein, Germany, 23538

Germany

[Not yet recruiting]
Hamburg, Germany, 20251

Hungary

[Not yet recruiting]
Budapest, Hungary, 1036

[Not yet recruiting]
Budapest, Hungary, 1089

[Not yet recruiting]
Budapest, Hungary, 1106

[Not yet recruiting]
Debrecen, Hungary, 4032

[Not yet recruiting]
Veszprem, Hungary, 8200

Italy

[Not yet recruiting]
Ancona, Italy, 60126

[Not yet recruiting]
Bari, Italy, 70124

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Catania, Italy, 95123

[Not yet recruiting]
Firenze, Italy, 50139

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Milano, Italy, 20122

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[Not yet recruiting]
Torino, Italy, 10128

Japan, Aichi

[Not yet recruiting]
Nagoya, Aichi, Japan, 466-8560

[Not yet recruiting]
Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

[Not yet recruiting]
Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

[Not yet recruiting]
Suita, Osaka, Japan, 565-0871

Japan, Tokyo

[Not yet recruiting]
Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

[Not yet recruiting]
Kyoto, Japan, 606-8507

Korea, Republic of, Korea

[Not yet recruiting]
Seoul, Korea, Korea, Republic of, 110 744

Korea, Republic of

[Not yet recruiting]
Ask Contact, Korea, Republic of

[Not yet recruiting]
Incheon, Korea, Republic of, 405-760

[Not yet recruiting]
Kungki-do, Korea, Republic of, 463-707

[Not yet recruiting]
Seoul, Korea, Republic of, 110-744

[Not yet recruiting]
Seoul, Korea, Republic of, 138-736

Latvia

[Not yet recruiting]
Riga, Latvia, 1009

[Not yet recruiting]
Riga, Latvia, LV-1002

Singapore

[Not yet recruiting]
Ask Contact, Singapore, 168751

[Not yet recruiting]
Singapore, Singapore, 119074

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ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

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3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
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16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
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22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

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Study NCT01012973
on Date: November 29, 2011 (v16)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	2009-010973-19 [EudraCT Number]

Study Status

Record Verification:	November 2011
Overall Status:	Active, not recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Actual]
Study Completion:	March 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	November 29, 2011

Last Update Posted:	November 30, 2011 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	177 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Biological: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 52 every 4 weeks plus additional on week 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; week 52 VEGF Trap-Eye injection (unless investigator declines for medical reasons), weeks 60 and 68 re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; last visit (no treatment) at week 76.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5.

Change in the NEI-VFQ-25 total score from baseline
Week 24

6. Change in the EQ-5D score from baseline
Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness ≥ 250 µm on optical coherence tomography (OCT)
- Adults ≥ 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1

- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66421

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Marburg, Germany, 35037

Hungary

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Budapest, Hungary, 1133

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

Japan, Aichi

Nagoya, Aichi, Japan, 466-8560

Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

Suita, Osaka, Japan, 565-0871

Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

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ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

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5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
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12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: December 18, 2012 (v20)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	2009-010973-19 [EudraCT Number]

Study Status

Record Verification:	December 2012
Overall Status:	Completed
Study Start:	October 2009
Primary Completion:	February 2011 [Actual]
Study Completion:	February 2012 [Actual]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Results First Submitted:	October 23, 2012
	October 23, 2012

Results First Submitted that Met QC Criteria:	
Results First Posted:	November 22, 2012 [Estimate]
Certification/Extension First Submitted:	January 26, 2012
Certification/Extension First Submitted that Met QC Criteria:	January 26, 2012
Certification/Extension First Posted:	January 30, 2012 [Estimate]
Last Update Submitted that Met QC Criteria:	December 18, 2012
Last Update Posted:	December 19, 2012 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	

Macular Edema
Central Retinal Vein Occlusion
CRVO
VEGF Trap-Eye
best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	177 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.	Biological: Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321) Intravitreal injection. Weeks 0 to 20 of Aflibercept Injection every 4 weeks; Weeks 24 to 52 every 4 weeks PRN (pro re nata, on demand); plus additional on Week 60 and 68.
Sham Comparator: Sham treatment Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept	Sham treatment Sham treatment. Weeks 0 to 52 sham treatment every 4 weeks; plus additional on Week 60 and 68. Biological: Sham treatment switched to Aflibercept Injection

Arms	Assigned Interventions
Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.	Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Outcome Measures

[See Results Section]

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness ≥ 250 µm on optical coherence tomography (OCT)
- Adults ≥ 18 years
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye

Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye

- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

Study Officials: Bayer Study Director
Study Director
Bayer

Locations: **Australia, New South Wales**

Chatswood, New South Wales, Australia, 2067

Parramatta, New South Wales, Australia, 2150

Sydney, New South Wales, Australia, 2000

Westmead, New South Wales, Australia, 2145

Australia, Victoria

East Melbourne, Victoria, Australia, 3002

Australia, Western Australia

Nedlands, Western Australia, Australia, 6009

Austria

Innsbruck, Austria, 6020

Linz, Austria, 4021

Wien, Austria, 1090

France, Cedex 12

Paris, Cedex 12, France, 75557

France, Cedex 1

Nantes, Cedex 1, France, 44093

France

Bordeaux, France, 33000

Dijon, France, 21033

Marseille, France, 13008

Paris, France, 75015

Germany, Baden-Württemberg

Freiburg, Baden-Württemberg, Germany, 79106

Heidelberg, Baden-Württemberg, Germany, 69120

Tübingen, Baden-Württemberg, Germany, 72076

Germany, Bayern

München, Bayern, Germany, 81675

Regensburg, Bayern, Germany, 93053

Germany, Hessen

Darmstadt, Hessen, Germany, 64297

Frankfurt, Hessen, Germany, 60596

Marburg, Hessen, Germany, 35037

Germany, Niedersachsen

Göttingen, Niedersachsen, Germany, 37075

Germany, Nordrhein-Westfalen

Aachen, Nordrhein-Westfalen, Germany, 52074

Bonn, Nordrhein-Westfalen, Germany, 53105

Essen, Nordrhein-Westfalen, Germany, 45122

Köln, Nordrhein-Westfalen, Germany, 50924

Münster, Nordrhein-Westfalen, Germany, 48145

Germany, Rheinland-Pfalz

Ludwigshafen, Rheinland-Pfalz, Germany, 67063

Mainz, Rheinland-Pfalz, Germany, 55131

Germany, Saarland

Homburg, Saarland, Germany, 66421

Germany, Sachsen

Chemnitz, Sachsen, Germany, 09116

Dresden, Sachsen, Germany, 01307

Dresden, Sachsen, Germany, 06067

Leipzig, Sachsen, Germany, 04103

Germany, Schleswig-Holstein

Kiel, Schleswig-Holstein, Germany, 24105

Lübeck, Schleswig-Holstein, Germany, 23538

Germany

Berlin, Germany, 13353

Hamburg, Germany, 20251

Hungary

Budapest, Hungary, 1089

Budapest, Hungary, 1106

Budapest, Hungary, 1133

Debrecen, Hungary, 4032

Veszprem, Hungary, 8200

Zalaegerszeg, Hungary, H-8900

Italy

Ancona, Italy, 60126

Bari, Italy, 70124

Catania, Italy, 95123

Firenze, Italy, 50134

Milano, Italy, 20122

Milano, Italy, 20132

Milano, Italy, 20157

Padova, Italy, 35128

Roma, Italy, 00133

Roma, Italy, 00198

Torino, Italy, 10122

Japan, Aichi

Nagoya, Aichi, Japan, 466-8560

Nagoya, Aichi, Japan, 467-8602

Japan, Chiba

Urayasu, Chiba, Japan, 279-0021

Japan, Osaka

Suita, Osaka, Japan, 565-0871

Japan, Tokyo

Chiyoda-ku, Tokyo, Japan, 101-8309

Japan

Kyoto, Japan, 606-8507

Korea, Republic of

Incheon, Korea, Republic of, 405-760

Kungki-do, Korea, Republic of, 463-707

Seoul, Korea, Republic of, 110 744

Seoul, Korea, Republic of, 110-744

Seoul, Korea, Republic of, 138-736

Seoul, Korea, Republic of

Latvia

Riga, Latvia, 1002

Riga, Latvia, 1050

Singapore

Singapore, Singapore, 119074

Singapore, Singapore, 168751

IPDSharing

Plan to Share IPD:

References

Citations:

Links:

Available IPD/Information:

Study Results

Participant Flow

Reporting Groups

	Description
Aflibercept Injection First, Then Aflibercept Injection	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment First, Then Aflibercept Injection	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Overall Study

	Aflibercept Injection First, Then Aflibercept Injection	Sham Treatment First, Then Aflibercept Injection
Started	106	71
Participants Received Treatment	104 [1]	68 [1]
Fulfilled Requirements of FAS Population	103 [2]	68 [2]
Completed Week 24, From FAS	97	57
Completed Week 52, From FAS	91	52
Completed	90	52
Not Completed	16	19
Adverse Event	5	5
Lack of Efficacy	0	5

	Aflibercept Injection First, Then Aflibercept Injection	Sham Treatment First, Then Aflibercept Injection
Lost to Follow-up	1	0
(Overseas travel - indefinite period)	1	0
Increase in vis. acuity, never injected	0	1
Protocol Violation	5	2
Withdrawal by Subject	4	6

[1] Safety Population: Participants received treatment

[2] Full Analysis Set (FAS) Population: Participants received treatment with post baseline measurements

Baseline Characteristics

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Baseline Measures

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Overall Number of Participants		104	68	172
Age Continuous Mean (Standard Deviation) Unit of measure: Years	Number Analyzed	104 Participants	68 Participants	172 Participants
		60.0 (12.3)	63.8 (13.3)	61.5 (12.8)
Sex: Female, Male Measure type: Count of Participants Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
	Female	45 43.27%	31 45.59%	76 44.19%
	Male	59 56.73%	37 54.41%	96 55.81%
Ethnicity (NIH/OMB) Measure type: Count of Participants Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
	Hispanic or Latino	4 3.85%	1 1.47%	5 2.91%
	Not Hispanic or Latino	100 96.15%	66 97.06%	166 96.51%
	Unknown or Not Reported	0 0%	1 1.47%	1 0.58%
Baseline Best Corrected Visual Acuity (BCVA) letter scores [1] Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		53.5 (15.7)	50.9 (15.4)	52.5 (15.6)

[1] Only participants with a ETDRS (Early Treatment Diabetic Retinopathy Study) Best Corrected Visual Acuity letter score of 73 to 25 (= Acuity of 20/40 to 20/320) in the study eye at 4 meters were included, Safety Analysis Set (EYLEA n=104, Sham n=68); a higher score represents better functioning.

		104 Participants	68 Participants	172 Participants

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Number of participants with baseline retinal perfusion [1] Measure type: Number Unit of measure: Participants	Number Analyzed			
Perfused		90	54	144
Nonperfused		7	7	14
Indeterminate		7	7	14
	[1] Retinal perfusion defined as less than 10 disc areas of capillary nonperfusion using fluorescein angiography (FA)			
Baseline Retinal Thickness by Optical Coherence Tomography (OCT) Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		682.78 (233.36)	638.66 (224.69)	665.34 (230.33)
Baseline intraocular pressure Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		15.2 (2.8)	14.4 (2.7)	14.9 (2.8)
Number of participants with time since Central retinal vein occlusion (CRVO) diagnosis Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
>= 2 months		46	33	79
< 2 months		56	35	91
Missing		2	0	2

		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment	Total
Baseline National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) total score [1]	Number Analyzed	104 Participants	68 Participants	172 Participants
		79.66 (13.06)	78.94 (14.00)	79.38 (13.40)

[1] The NEI VFQ-25 total score ranges from 0-100 with a score of 0 being the worst outcome and 100 being the best outcome. The NEI VFQ questionnaire is organized as a collection of subscales which are all scored from 0-100. To reach the overall composite score, each sub-scale score is averaged in order to give each sub-scale equal weight.

European questionnaire 5 dimensions (EQ-5D) total score [1] Mean (Standard Deviation)	Number Analyzed	104 Participants	68 Participants	172 Participants
		0.87 (0.15)	0.86 (0.16)	0.87 (0.15)

[1] The EQ-5D total score ranges from -0.594 to 1.000 with -0.594 being the worst.

Race Measure type: Number Unit of measure: Participants	Number Analyzed	104 Participants	68 Participants	172 Participants
Asian		26	15	41
White		75	49	124
Unknown or Not Reported		3	4	7

Outcome Measures

1. Primary Outcome Measure:

Measure Title	

	Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures
Measure Description	Defined study baseline range of Early Treatment Diabetic Retinopathy Study (ETDRS) Best Corrected Visual Acuity (BCVA) letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. Nominator = (Number of participants who maintained vision * 100); Denominator = Number of participants analyzed.
Time Frame	Baseline and Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures Measure Type: Number Unit of Measure: Percentage of participants	60.2	22.1

Statistical Analysis 1 for Percentage of Participants Who Gained at Least 15 Letters in BCVA as Measured by ETDRS Letter Score Compared With Baseline at Week 24 With Discontinued Participants Before Week 24 Evaluated as Failures

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis of difference of Eylea minus Sham of 0 was tested. In the database close after Week 24, basis for primary efficacy evaluation, 56 Sham / 96 Eylea subjects were considered as week 24 completers.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	[Not specified]
	Method	Cochran-Mantel-Haenszel
	Comments	[Not specified]

	Estimation Parameter	CMH adjusted difference

Method of Estimation	Estimated Value	38.3
	Confidence Interval	(2-sided) 95% 24.4 to 52.1
	Estimation Comments	The estimate is calculated as Eylea minus Sham. A positive value shows Eylea showed a higher BCVA total score compared to Sham.

2. Secondary Outcome Measure:

Measure Title	Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF)
Measure Description	Defined study baseline range of ETDRS Best Corrected Visual Acuity letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. However, because this was assessed at the screening visit, subjects may have had a higher BCVA recorded at the baseline visit and would not have been excluded from the study.
Time Frame	Baseline and Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI

	depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF) Measure Type: Mean (Standard Deviation) Unit of Measure: Letters correctly read	71.6 (17.1)	54.3 (20.2)

Statistical Analysis 1 for Change From Baseline in BCVA as Measured by Early Treatment Diabetic Retinopathy Study (ETDRS) Letter Score at Week 24 - Last Observation Carried Forward (LOCF)

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis was equality in change from baseline to Week 24 in BCVA total letter score between Eylea and Sham. If primary efficacy was successful, secondary efficacy endpoints were tested in a pre-specified fixed sequence testing procedure. Change in BCVA letter

		score was to be tested first in this sequence.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	As primary efficacy evaluation was significant, and this p-value was below significance level of two-sided <.05, the fixed sequence testing did continue with next secondary endpoint.
	Method	ANOVA
	Comments	ANOVA, adjusting for region and baseline BCVA category as fixed factors.

Method of Estimation	Estimation Parameter	Difference in Least square means
	Estimated Value	14.7
	Confidence Interval	(2-sided) 95% 10.8 to 18.7
	Estimation Comments	The difference is calculated as Eylea minus Sham. A positive value indicates Eylea showed a higher change in BCVA total score until week 24 compared to Sham.

3. Secondary Outcome Measure:

Measure Title	Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF
Measure Description	
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	67
Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: microns	-448.58 (256.02)	-169.27 (224.72)

Statistical Analysis 1 for Change From Baseline in Central Retinal Thickness (CRT) at Week 24 - LOCF

	Comparison Groups	

Statistical Analysis Overview		Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Null hypothesis was equality in change from baseline to Week 24 in central retinal thickness between Eylea and Sham. If primary efficacy was successful, secondary efficacy end points were to be tested in a pre-specified fixed sequence testing procedure. Change in central retinal thickness was to be tested at second place in this sequence.
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	<.0001
	Comments	As fixed sequence testing did reject nullhypothesis of change from baseline in BCVA until week 24, and this p-value was below significance level of two-sided <.05, the fixed sequence testing did continue with next secondary endpoint.
	Method	ANCOVA
	Comments	ANCOVA, stratified by region and baseline BCVA category, baseline central retinal thickness added as covariate.

Method of Estimation	Estimation Parameter	Difference in Least square (LS) means
	Estimated Value	-239.42
	Confidence Interval	(2-sided) 95% -286.31 to -192.53

	Estimation Comments	The difference is calculated as Eylea minus Sham. A negative value indicates Eylea showed a higher reduction in change in central retinal thickness until week 24 compared to Sham.

4. Secondary Outcome Measure:

Measure Title	Percentage of Participants Who Developed Neovascularization During the First 24 Weeks
Measure Description	Formation of blood vessels in the anterior segment, optic disc, or elsewhere in the fundus up to Week 24
Time Frame	From baseline until Week 24

Analysis Population Description

Full analysis set

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	103	68
Percentage of Participants Who Developed Neovascularization During the First 24 Weeks Measure Type: Number Unit of Measure: Percentage of participants		
Any neovascularization	2.9	4.4
Anterior segment neovascularization	1.9	1.5
Neovascularization of the optic disc (NVD)	0.0	0.0
Neovascularization elsewhere in the fundus (NVE)	1.0	2.9

Statistical Analysis 1 for Percentage of Participants Who Developed Neovascularization During the First 24 Weeks

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	Nullhypothesis of no difference in development of neovascularizations between Eylea and Sham group was tested. (Any neovascularization)
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Statistical Test of Hypothesis	P-Value	0.5947
	Comments	As fixed sequence testing did reject nullhypothesis of change from baseline in CRT until week 24, and this p-value was not below significance level of two-sided <.05,

		the fixed sequence testing did end with this evaluation.
	Method	Cochran-Mantel-Haenszel
	Comments	Cochrane-Mantel-Haenszel test, stratified by region and baseline BCVA category.

Method of Estimation	Estimation Parameter	CMH adjusted Difference
	Estimated Value	-1.5
	Confidence Interval	(2-sided) 95% -7.4 to 4.4
	Estimation Comments	[Not specified]

5. Secondary Outcome Measure:

Measure Title	Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF
Measure Description	The NEI VFQ-25 total score ranges from 0-100 with a score of 0 being the worst outcome and 100 being the best outcome. The NEI VFQ questionnaire is organized as a collection of subscales which are all scored from 0-100. To reach the overall composite score, each sub-scale score is averaged in order to give each sub-scale equal weight
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often

	as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment, who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	96	65
Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: Scores on a scale	7.46 (9.55)	3.55 (9.74)

Statistical Analysis 1 for Change From Baseline in National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) Total Score at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	[Not specified]
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

	Estimation Parameter	Difference in LS means

Method of Estimation	Estimated Value	4.2
	Confidence Interval	(2-sided) 95% 1.7 to 6.8
	Estimation Comments	As the fixed sequence of secondary endpoints stopped with proportion of neovascularizations developed until week 24, 95% confidence interval is only of descriptive nature.

6. Secondary Outcome Measure:

Measure Title	Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF
Measure Description	EQ-5D is a quality of life questionnaire based on a scale from -0.594 (worst) to 1.00 (best).
Time Frame	Baseline and Week 24

Analysis Population Description

Full-Analysis Set with assessment for this outcome measure; imputation technique: LOCF

Reporting Groups

	Description
Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20, later as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Follow-up phase: Participants on IAI, who continued the study, received 2 mg dose of IAI depending on the study retreatment criteria at Week 60 and 68.
Sham Treatment	Participants received sham treatment administered every 4 weeks from Day 1 through Week 52. Follow-up phase: Participants on sham treatment,

	who switched to Intravitreal Aflibercept Injection (IAI), received a 2 mg dose of IAI at week 52 and depending on the study retreatment criteria at Week 60 and 68.

Measured Values

	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321)	Sham Treatment
Overall Number of Participants Analyzed	95	64
Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF Measure Type: Mean (Standard Deviation) Unit of Measure: Scores on a scale	0.029 (0.139)	-0.002 (0.195)

Statistical Analysis 1 for Change From Baseline in European Five-dimensional Health Scale (EQ-5D) Score at Week 24 - LOCF

Statistical Analysis Overview	Comparison Groups	Aflibercept Injection (EYLEA, VEGF Trap-Eye, BAY86-5321), Sham Treatment
	Comments	[Not specified]
	Type of Statistical Test	Superiority or Other (legacy)
	Comments	[Not specified]

Method of Estimation	Estimation Parameter	Difference in LS Means
	Estimated Value	0.044
	Confidence Interval	(2-sided) 95% -0.002 to 0.09
	Estimation Comments	As the fixed sequence of secondary endpoints stopped with proportion of neovascularizations developed until week 24, 95% confidence interval is only of descriptive nature.

Reported Adverse Events

Time Frame	[Not specified]
Adverse Event Reporting Description	[Not specified]

Reporting Groups

	Description
Aflibercept Injection (Until Week 20)	Participants received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered every 4 weeks from Day 1 through Week 20. Participants were observed until Week 24. Participants in the safety population were at risk.
Sham Treatment (Until Week 20)	Participants received sham treatment administered every 4 weeks from Day 1 through Week 20. Participants were observed until Week 24. Participants in the safety population were at risk.
Aflibercept Injection (Until Week 48)	Participants who continued the study drug until Week 24 received a 2 mg dose of Intravitreal Aflibercept Injection (IAI) administered as often as every 4 weeks depending on the study retreatment criteria from Week 24 through Week 48. Participants were observed from Week 24 until Week 52. Participants in the safety population that completed Week 24 were at risk.
Sham Treatment (Until Week 48)	Participants who continued the study drug until Week 24 received sham treatment administered every 4 weeks from Week 24 to Week 48. Participants were observed from Week 24 until Week 52. Participants in the safety population that completed Week 24 were at risk.
Aflibercept Injection Continued (Until Week 68)	Participants on IAI who continued the study drug until Week 52, received 2 mg dose of IAI depending on the study retreatment criteria at Week 52, 60 and 68. Participants were observed starting from Week 52. Participants in the safety population that completed Week 52 were at risk.

Sham Treatment Then Aflibercept Injection (Until Week 68)	Participants on sham treatment switched to IAI, received a 2 mg dose of IAI at Week 52 and depending on the study retreatment criteria at Week 60 and 68. Participants were observed starting from Week 52. Participants in the safety population that completed Week 52 were at risk.

All-Cause Mortality

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)
Total						

Serious Adverse Events

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)
Total	8/104 (7.69%)	8/68 (11.76%)	14/97 (14.43%)	7/57 (12.28%)	4/91 (4.4%)	3/52 (5.77%)
Cardiac disorders						
Aortic valve incompetence [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Cardiac failure [A*]						

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Coronary artery stenosis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Diastolic dysfunction [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Mitral valve incompetence [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Eye disorders						
Blindness unilateral [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Glaucoma [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Iris neovascularisation [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular fibrosis [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular ischaemia [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Macular oedema [A*]	0/104 (0%)	2/68 (2.94%)	4/97 (4.12%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Retinal vein occlusion [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Visual acuity reduced [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	0/57 (0%)	2/91 (2.2%)	0/52 (0%)
Vitreous detachment [A*]	1/104	0/68	0/97	0/57	0/91	0/52

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Vitreous haemorrhage [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Gastrointestinal disorders						
Diverticular perforation [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Hepatobiliary disorders						
Hepatic function abnormal [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Infections and infestations						
Furuncle [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Gastroenteritis [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Pneumonia [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Vestibular neuronitis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Injury, poisoning and procedural complications						
Fall [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Femur fracture [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Hand fracture [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Humerus fracture [A*]	0/104 (0%)	1/68 (1.47%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Radius fracture [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Spinal compression fracture [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Musculoskeletal and connective tissue disorders						
Intervertebral disc protrusion [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Spinal column stenosis [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)						
Breast cancer [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Oropharyngeal cancer stage unspecified [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Nervous system disorders						
Paraesthesia [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Syncope [A*]	0/104 (0%)	0/68 (0%)	1/97 (1.03%)	2/57 (3.51%)	0/91 (0%)	0/52 (0%)
Transient ischaemic attack [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Respiratory, thoracic and mediastinal disorders						
Dyspnoea [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	1/57 (1.75%)	0/91 (0%)	0/52 (0%)
Laryngeal granuloma [A*]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Pulmonary hypertension [A*]	0/104 (0%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	1/52 (1.92%)
Surgical and medical procedures						
Ischaemic heart disease prophylaxis [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vascular disorders						
Circulatory collapse [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	0/57 (0%)	0/91 (0%)	0/52 (0%)

* Indicates events were collected by non-systematic methods.

A Term from vocabulary, MedDRA (14.1)

Other Adverse Events

Frequency Threshold Above Which Other Adverse Events are Reported: 5%

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)	Affected/At Risk (%)
Total	52/104 (50%)	44/68 (64.71%)	66/97 (68.04%)	30/57 (52.63%)	38/91 (41.76%)	19/52 (36.54%)
Blood and lymphatic system disorders						
Anaemia [A*]	1/104 (0.96%)	0/68 (0%)	0/97 (0%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Eye disorders						
Conjunctival haemorrhage [A+]	10/104 (9.62%)	3/68 (4.41%)	3/97 (3.09%)	0/57 (0%)	9/91 (9.89%)	3/52 (5.77%)
Eye irritation [A+]	3/104 (2.88%)	7/68 (10.29%)	4/97 (4.12%)	1/57 (1.75%)	1/91 (1.1%)	2/52 (3.85%)
Eye pain [A+]	12/104 (11.54%)	3/68 (4.41%)	6/97 (6.19%)	2/57 (3.51%)	1/91 (1.1%)	0/52 (0%)
Foreign body sensation in eyes [A+]	6/104 (5.77%)	5/68 (7.35%)	2/97 (2.06%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Lacrimation increased [A+]	3/104 (2.88%)	4/68 (5.88%)	3/97 (3.09%)	4/57 (7.02%)	1/91 (1.1%)	2/52 (3.85%)
Macular fibrosis [A+]	1/104 (0.96%)	1/68 (1.47%)	5/97 (5.15%)	4/57 (7.02%)	0/91 (0%)	3/52 (5.77%)
Macular ischaemia [A+]	7/104 (6.73%)	5/68 (7.35%)	3/97 (3.09%)	1/57 (1.75%)	0/91 (0%)	1/52 (1.92%)
Macular oedema [A+]	2/104 (1.92%)	9/68 (13.24%)	30/97 (30.93%)	7/57 (12.28%)	17/91 (18.68%)	2/52 (3.85%)
Ocular hyperaemia [A+]	5/104 (4.81%)	4/68 (5.88%)	2/97 (2.06%)	1/57 (1.75%)	4/91 (4.4%)	1/52 (1.92%)
Optic disc vascular disorder [A+]	5/104 (4.81%)	3/68 (4.41%)	3/97 (3.09%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Retinal exudates [A+]	8/104 (7.69%)	5/68 (7.35%)	4/97 (4.12%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Retinal haemorrhage [A+]	4/104 (3.85%)	6/68 (8.82%)	11/97 (11.34%)	5/57 (8.77%)	5/91 (5.49%)	2/52 (3.85%)
Retinal vascular disorder [A+]	6/104	7/68	10/97	2/57	0/91	2/52

	Aflibercept Injection (Until Week 20)	Sham Treatment (Until Week 20)	Aflibercept Injection (Until Week 48)	Sham Treatment (Until Week 48)	Aflibercept Injection Continued (Until Week 68)	Sham Treatment Then Aflibercept Injection (Until Week 68)
Visual acuity reduced [A]	2/104 (1.92%)	7/68 (10.29%)	10/97 (10.31%)	1/57 (1.75%)	7/91 (7.69%)	1/52 (1.92%)
Vitreous detachment [A]	2/104 (1.92%)	1/68 (1.47%)	7/97 (7.22%)	0/57 (0%)	0/91 (0%)	0/52 (0%)
Vitreous floaters [A]	6/104 (5.77%)	0/68 (0%)	1/97 (1.03%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Gastrointestinal disorders						
Nausea [A]	0/104 (0%)	1/68 (1.47%)	0/97 (0%)	3/57 (5.26%)	0/91 (0%)	0/52 (0%)
Infections and infestations						
Influenza [A]	2/104 (1.92%)	0/68 (0%)	5/97 (5.15%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)
Nasopharyngitis [A]	8/104 (7.69%)	6/68 (8.82%)	10/97 (10.31%)	11/57 (19.3%)	4/91 (4.4%)	2/52 (3.85%)
Investigations						
Intraocular pressure increased [A]	9/104 (8.65%)	4/68 (5.88%)	14/97 (14.43%)	2/57 (3.51%)	2/91 (2.2%)	1/52 (1.92%)
Visual acuity tests abnormal [A]	0/104 (0%)	1/68 (1.47%)	5/97 (5.15%)	0/57 (0%)	1/91 (1.1%)	0/52 (0%)
Musculoskeletal and connective tissue disorders						
Arthralgia [A]	1/104 (0.96%)	5/68 (7.35%)	2/97 (2.06%)	1/57 (1.75%)	2/91 (2.2%)	0/52 (0%)
Nervous system disorders						
Headache [A]	7/104 (6.73%)	4/68 (5.88%)	4/97 (4.12%)	1/57 (1.75%)	1/91 (1.1%)	1/52 (1.92%)

	Afliberce Injection (Until Week 20)	Sham Treatme (Until Week 20)	Afliberce Injection (Until Week 48)	Sham Treatme (Until Week 48)	Afliberce Injection Continue (Until Week 68)	Sham Treatme Then Afliberce Injection (Until Week 68)
Vascular disorders						
Hypertension [A*]	4/104 (3.85%)	3/68 (4.41%)	4/97 (4.12%)	4/57 (7.02%)	3/91 (3.3%)	2/52 (3.85%)

* Indicates events were collected by non-systematic methods.

A Term from vocabulary, MedDRA (14.1)

Limitations and Caveats

[Not specified]

More Information

Certain Agreements:

Principal Investigators are NOT employed by the organization sponsoring the study.

There IS an agreement between the Principal Investigator and the Sponsor (or its agents) that restricts the PI's rights to discuss or publish trial results after the trial is completed.

Publishing of result communication only after Bayer´s written approval. Manuscript to Bayer sixty days before public release. If no written Bayer comment within 60 days consider approval given. If multi-site study, principal investigator (PI) not do independently publish results before publication of the multi-site paper, but PI not restricted from 24 months from study to completion onwards.

Results Point of Contact:

Name/Official Title: Therapeutic Area Head
Organization: BAYER
Phone:
Email: clinical-trials-contact@bayerhealthcare.com

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ClinicalTrials.gov archive

History of Changes for Study: NCT01012973

Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)

Latest version (submitted October 27, 2014) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's date link to see a rendering of the study for that version.
- Edits or deletions will be displayed in ~~red~~.
- Additions will be displayed in *green*.
- The yellow choices in the table indicate the study versions currently compared below. A yellow row indicates the study version being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	◉	○	November 12, 2009	Nothing (earliest Version on record)
2	○	○	January 21, 2010	Contacts/Locations, Study Status, Study Identification and Study Description
3	○	○	February 9, 2010	Contacts/Locations and Study Status
4	○	○	March 16, 2010	Contacts/Locations, Study Status and Study Identification
5	○	○	April 16, 2010	Contacts/Locations, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
6	○	○	July 22, 2010	Contacts/Locations, Study Status, Eligibility and Arms and Interventions
7	○	○	August 25, 2010	Study Status and Contacts/Locations
8	○	○	August 26, 2010	Recruitment Status, Study Status and Contacts/Locations
9	○	○	September 8, 2010	Study Status
10	○	○	October 4, 2010	Study Status
11	○	○	November 1, 2010	Study Status
12	○	○	January 25, 2011	Study Status and Contacts/Locations
13	○	○	April 8, 2011	Study Status and Study Design
14	○	○	June 23, 2011	Arms and Interventions, Study Status, Contacts/Locations and Eligibility
15	○	○	September 19, 2011	Study Status
16	○	○	November 29, 2011	Study Status and Study Identification
17	○	○	January 26, 2012	Study Status and Contacts/Locations
18	○	○	February 20, 2012	Recruitment Status, Study Status
19	○	○	October 23, 2012	Outcome Measures, Arms and Interventions, Study Status, More Information, Reported Adverse Events, Baseline Characteristics and Participant Flow
20	○	○	December 18, 2012	More Information, Arms and Interventions, Study Status and Baseline Characteristics
21	○	○	January 18, 2013	Arms and Interventions, More Information, Study Status and Baseline Characteristics
22	○	○	January 30, 2014	Contacts/Locations, Sponsor/Collaborators, More Information, Study Status, Baseline Characteristics and References
23	○	◉	October 27, 2014	More Information, Study Status and References

Compare | Comparison Format: (•) Merged ( ) Side-by-Side

Scroll up to access the controls

Study NCT01012973
on Date: January 21, 2010 (v2)

Study Identification

Unique Protocol ID:	14130
Brief Title:	Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO) (GALILEO)
Official Title:	A Randomized, Double-masked, Sham-controlled Phase 3 Study of the Efficacy, Safety and Tolerability of Repeated Intravitreal Administration of VEGF Trap-Eye in Subjects With Macular Edema Secondary to Central Retinal Vein Occlusion (CRVO)
Secondary IDs:	EudraCT: 2009-010973-19

Study Status

Record Verification:	January 2010
Overall Status:	Recruiting
Study Start:	October 2009
Primary Completion:	February 2011 [Anticipated]
Study Completion:	August 2012 [Anticipated]
First Submitted:	October 30, 2009
First Submitted that Met QC Criteria:	November 12, 2009
First Posted:	November 13, 2009 [Estimate]
Last Update Submitted that Met QC Criteria:	January 21, 2010

Last Update Posted:	January 22, 2010 [Estimate]

Sponsor/Collaborators

Sponsor:	Bayer
Responsible Party:	
Collaborators:	Regeneron Pharmaceuticals

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	Yes

Study Description

Brief Summary:	To determine the efficacy of vascular endothelial growth factor (VEGF) Trap-Eye injected into the eye on vision function in subjects with macular edema as a consequence of central retinal vein occlusion
Detailed Description:	

Conditions

Conditions:	Retinal Vein Occlusion
Keywords:	Macular Edema Central Retinal Vein Occlusion CRVO VEGF Trap-Eye best-corrected visual acuity

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 3
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	TripleParticipant, Investigator, Outcomes Assessor
Allocation:	Randomized
Enrollment:	165 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Arm 1	Drug: VEGF Trap-Eye (BAY86-5321) Intravitreal injection. Weeks 0 to 20 injection of VEGF Trap-Eye every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and either (PRN) injection of VEGF Trap-Eye or sham injection; weeks 52 to 100 safety follow-up.
Sham Comparator: Arm 2	Sham treatment Sham treatment. Weeks 0 to 20 sham treatment every 4 weeks; weeks 24 to 48 every 4 weeks re-assessment and sham injection; weeks 52 to 100 safety follow-up.

Outcome Measures

Primary Outcome Measures:

1. The proportion of subjects who gain at least 15 letters in BCVA on the EDTRS chart compared with baseline at the Week 24 endpoint
   Week 24

Secondary Outcome Measures:

2. Change from baseline in BCVA score
   Week 24
3. Absolute change from baseline in central retinal thickness, assessed by OCT
   Week 24
4. Proportion of subjects progressing to anterior segment neovascularization, neovascularization of the optic disc (NVD), or neovascularization of the retina elsewhere (NVE) requiring pan-retinal photocoagulation
   Week 24
5. Change in the NEI-VFQ-25 total score from baseline
   Week 24
6. Change in the EQ-5D score from baseline
   Week 24

Eligibility

Minimum Age: 18 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) for no longer than 9 months with mean central subfield thickness >= 250 µm on optical coherence tomography (OCT).
- Adults >= 18 years.
- early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye.

Exclusion Criteria:

- Previous treatment with anti-angiogenic drugs in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.)
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye

Contacts/Locations

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Study Officials:

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Australia, Western Australia

[Recruiting]
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Singapore, Singapore, 119074

[Not yet recruiting]
Singapore, Singapore, 168751

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Electronic Acknowledgement Receipt	
EFS ID:	38658587
Application Number:	16397267
International Application Number:	
Confirmation Number:	8135
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-008CIPCON5
Receipt Date:	21-FEB-2020
Filing Date:	29-APR-2019
Time Stamp:	15:49:41
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	0725US06_2020-02-21_Supp_IDS_trans_REGN-008CIPCON5.pdf	50616 e578c05302b0e045db959d29256dfcd642590263	no	2

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Information:					
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		Total Files Size (in bytes):	5719844		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronically Filed

INFORMATION DISCLOSURE STATEMENT	Attorney Docket No.	REGN-008CIPCON5
	Confirmation No.	8135
	First Named Inventor	George D. Yancopoulos
	Application Number	16/397,267
	Filing Date	April 29, 2019
	Group Art Unit	
	Examiner Name	
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:

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

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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-008CIPCON5.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 21 February 2020

By: /Karl Bozicevic, Reg. No. 28,807/
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	George D. Yancopoulos
				Art Unit	
				Examiner Name	
Sheet	1	of	2	Attorney Docket Number	REGN-008CIPCON5

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

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

FOREIGN PATENT DOCUMENTS

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	George D. Yancopoulos
				Art Unit	
				Examiner Name	
Sheet	2	of	2	Attorney Docket Number	REGN-008CIPCON5

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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		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.

REVIEW of OPHTHALMOLOGY

Christopher Kent, Senior Editor

PUBLISHED 5 AUGUST 2019

Anti-VEGF 2019: The State of the Art

Surgeons share the latest thinking about the benefits and risks of anti-VEGF therapy, and what lies ahead.

There's no question that anti-VEGF drugs have caused a sea-change in the treatment of several retinal diseases. Conditions that were basically untreatable have now become manageable, preventing what was once almost inevitable blindness. Yet there's still plenty of room for improvement; results vary, and not all patients avoid vision loss.

"When I started my career, neovascular age-related macular degeneration was a horrible disease," recalls K. Bailey Freund, MD, a retina specialist and a clinical professor of ophthalmology at the New York University School of Medicine. (Dr. Freund has been a principal investigator in several pivotal trials of novel treatments for retinal diseases.) "Nothing we could do would really help our patients. Today, certain patients do extremely well—particularly the ones that have Type 1 (subretinal pigment epithelium) neovascularization under the fovea. Others do well initially, but eventually the non-neovascular aspect of the disease kicks in and they ultimately end up losing vision, more from macular atrophy than exudative complications."

Here, retina specialists answer key questions about the current state of anti-VEGF drug treatments for macular degeneration and other retinal diseases; share the latest insights, which suggest that neovascularization and subretinal fluid are not always a bad thing; and discuss what the upcoming years may hold.

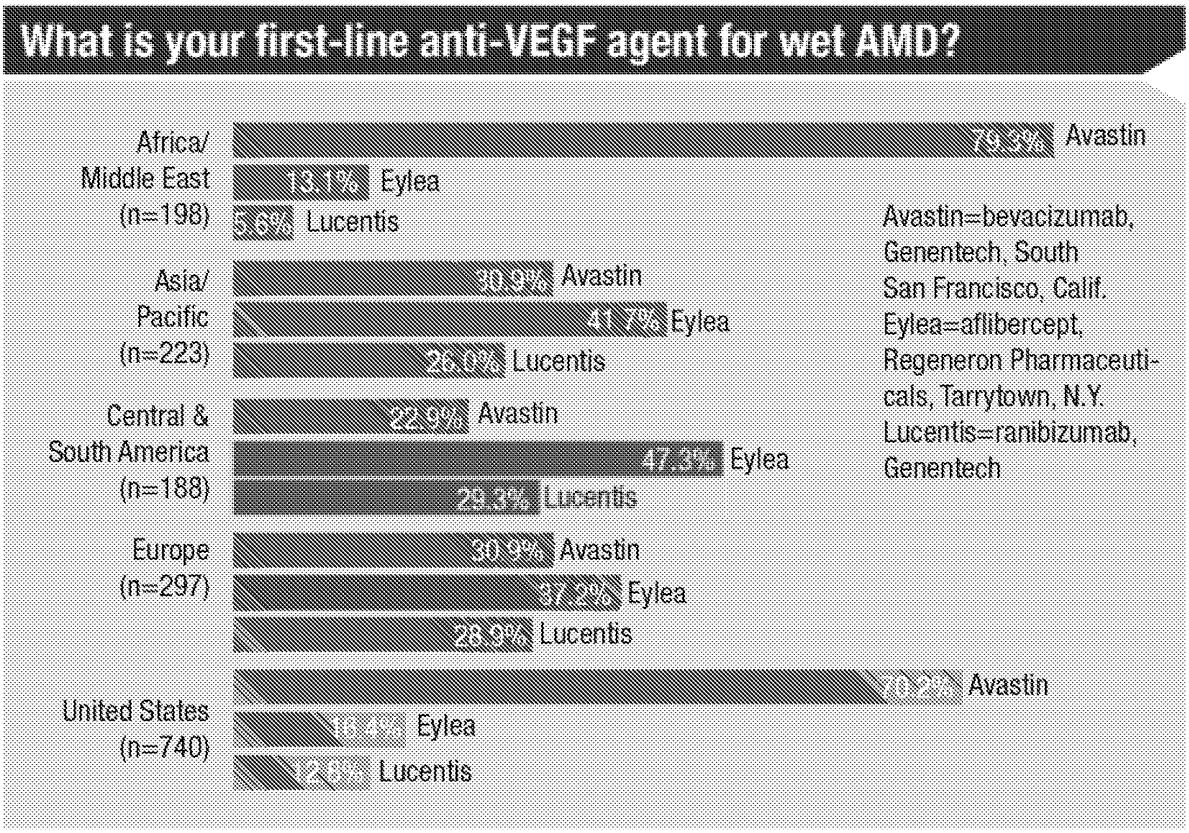
Anti-VEGF Drug Choice

"Each anti-VEGF option has an advantage that certain people prefer," notes Philip Rosenfeld, MD, PhD, professor of ophthalmology at the Bascom Palmer Eye Institute at the University of Miami Miller School of Medicine. "For example, bevacizumab is cheap. In certain countries surgeons can get a rebate for using ranibizumab, so some doctors use it because it's financially rewarding for the practice. Aflibercept is the preferred drug for certain neovascular types of disease; for example, I think it works best in fibrovascular retinal pigment epithelial detachments with a significant serous component.

"Personally, I treat with bevacizumab and aflibercept," he says. "In my non-hospital-based setting, I always start with bevacizumab and try to get a durability that exceeds six, eight or 10 weeks. If I can't get beyond that six-to-eight week interval, then I switch to aflibercept. Some surgeons prefer ranibizumab, and others prefer to use aflibercept exclusively. However, many times when patients are in Medicare Advantage plans, the choice of drug is decided by the provider."

Mylan Exhibit 1017
Mylan v. Regeneron, IPR2022-01226
Page 576

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Data from the Global Trends in Retina Survey, conducted by the American Society of Retina Specialists, in conjunction with the 20th Annual ASRS Preferences and Trends (PAT) Survey.[3] Members of 42 retina societies around the world participated in the 2018 Global Trends in Retina Survey. (Reprinted with permission.)

Dr. Rosenfeld notes that The American Society of Retina Specialists runs an annual Patterns and Trends survey, in which they ask about anti-VEGF drug choices every year. “We don’t have the results for 2019 as we speak,” he says, “but the replies for 2018 indicated that in the U.S., bevacizumab is the first-line choice for treating wet AMD for about 70 percent of retina specialists. Ranibizumab is first-line for 12 percent, and aflibercept is first-line for 16 percent. Outside the U.S.—except in Africa and the Middle East—it looks like aflibercept wins. However, treating physicians are litigating for access to bevacizumab in the U.K. In France, it’s still very hard to get.” (He notes that at a recent retina meeting in Italy, most treating physicians said that they’ve used all three drug options—ranibizumab, bevacizumab and aflibercept.)

Dr. Freund says he finds the current anti-VEGF agents to be fairly similar in terms of efficacy and safety, so he chooses his drug regimen based on the individual patient’s presentation. “I might choose one drug over another when a patient is somewhat refractory to treatment,” he says. “This is particularly true for eyes that have what I currently refer to as aneurysmal Type 1 neovascularization, more commonly known as polypoidal choroidal vasculopathy. I see many patients with this lesion growth pattern because it was first described in our practice, and I’ve published on it extensively.

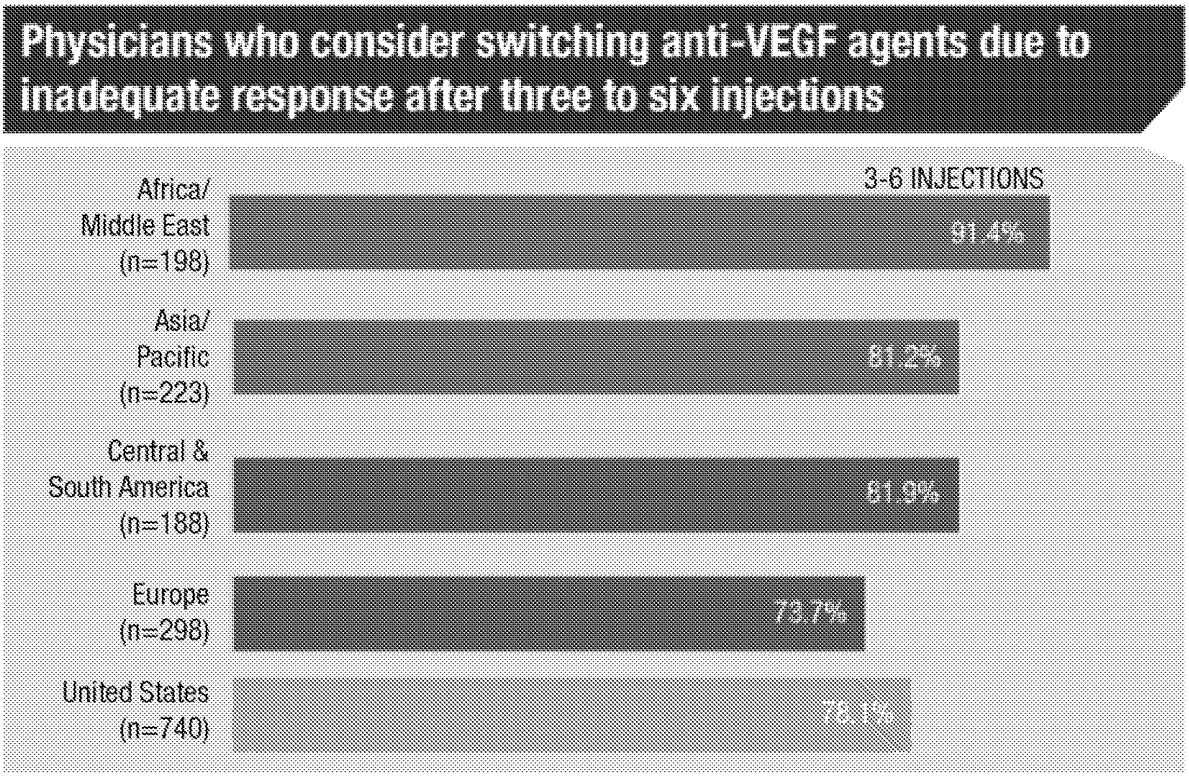
“Eyes with this lesion growth pattern may have a more robust response to aflibercept,” he continues. “Clinical trial evidence from the PLANET study showed that many of these eyes respond well to aflibercept monotherapy, while data from the EVEREST II trial indicates that eyes with aneurysmal Type 1 neovascularization receiving

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ranibizumab may require the addition of photodynamic therapy in order to optimize visual outcomes.

"There's another form of neovascular AMD that I call pachychoroid neovasculopathy," he notes. "These eyes have choroidal findings that are similar to those seen in eyes with central serous chorioretinopathy. Also, they lack some of the characteristic clinical findings we associate with typical macular degeneration in elderly Caucasian patients, such as soft drusen. Those eyes may benefit from aflibercept, since aflibercept has been shown to decrease choroidal thickness more than the other agents. I find that eyes which are somewhat refractory to anti-VEGF therapy may not respond as well to bevacizumab, compared to the other agents.



More data from the 2018 Global Trends in Retina Survey, conducted by the American Society of Retina Specialists.[3] (For more information, see the caption for the chart on page 27.)

"In the final analysis, a typical patient responds well to all of these agents," he says. "Numerous studies show that bevacizumab is noninferior to the alternative FDA-approved options. However, for patients managed on a treat-and-extend regimen, I'm less comfortable extending my dosing interval beyond eight weeks with bevacizumab. So, for patients with no out-of-pocket drug expense, we may choose a different agent that could require fewer injections, particularly when frequent office visits would be very difficult."

Of course, some patients don't respond to the first drug the treating physician tries, but Dr. Freund points out that switching refractory patients to an alternative anti-VEGF is not a cure-all. "When aflibercept was approved, many practices did what we then called 'switcher' studies," notes Dr. Freund. "We took patients who were poorly controlled with monthly bevacizumab or ranibizumab and switched them to aflibercept, expecting that because most eyes in the aflibercept trials could be maintained on injections every eight weeks, the same might happen with these refractory cases. But the eyes we were switching were very different from the type of newly-diagnosed cases

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enrolled in clinical trials. All we typically found was that aflibercept might get rid of more fluid for a little longer than the prior agents. We did not find that eyes showing persistent fluid with monthly ranibizumab were fluid-free for eight weeks with aflibercept."

Regarding the treatment protocol, Dr. Rosenfeld says he believes that most clinicians treat their patients using a treat-and-extend or modified treat-and-extend regimen. "This usually means performing monthly dosing until the macular fluid is resolved, and then extending the interval until macular fluid recurs," he explains. "At that point the interval is shortened, and eventually a treatment interval is defined for that particular patient. When I attended the FLORetina meeting in Florence, Italy recently, this protocol seemed to be the general consensus among the attendees."

Is Neovascularization Bad?

Clearly, neovascularization can be part of the problem in retinal diseases such as macular degeneration. However, many researchers note that anti-VEGF drugs aren't really treating the neovascularization—and they're beginning to suspect that neovascularization may actually be a good thing in some patients.

"There are three types of macular neovascularization," Dr. Rosenfeld explains. "The major type is what's called Type 1, which occurs under the retinal pigment epithelium. Today, with OCTA, particularly swept-source OCTA, we can identify Type 1 neovascularization long before exudation occurs. For that reason there's been some debate about whether we should treat Type 1 lesions with anti-VEGF therapy before exudation starts.

"I think the consensus is that we should not," he says. "Instead, we should watch the lesions and treat when symptomatic exudation develops. The reason is that those lesions don't go away because of treatment. What we're doing is training them not to leak. We calm the lesion down, but we don't make it go away.

Do Anti-VEGF Drugs Cause Atrophy?

Philip Rosenfeld, MD, PhD, professor of ophthalmology at the Bascom Palmer Eye Institute at the University of Miami Miller School of Medicine, believes the concern about anti-VEGF therapy possibly promoting the formation of macular atrophy is still present, but receding. "There's still some debate about whether the drugs themselves are influencing the formation of macular atrophy," he notes, "or whether it's just normal disease progression, or whether it's a characteristic of certain lesion types, such as Type 3 macular neovascularization, also known as retinal angiomatous proliferation, which tends to form atrophy."

K. Bailey Freund, MD, a retina specialist and a clinical professor of ophthalmology at the New York University School of Medicine, admits that a connection between anti-VEGF treatment and atrophy is biologically plausible. "Continuous VEGF suppression could do that, but we're not actually providing continuous suppression," he points out. "We give a large bolus of the drug, and over time the concentration drops to a very low level. Then we give another bolus. This may be one reason that the studies suggesting a possible link between anti-VEGF therapy and atrophy have really only shown this association when patients were treated continuously every month. Even these studies haven't really proven that the anti-VEGF treatment is causing the atrophy."

Dr. Freund advises clinicians to use their judgment. "Look at the data and use common sense," he says. "It might be reasonable to reduce injection frequency for eyes with Type 3 lesions, but not in eyes with large vascularized pigment epithelial detachments, particularly those with aneurysms—more commonly known as polyps—as these eyes appear resistant to macular atrophy, but are at risk for catastrophic hemorrhages if undertreated. So, look closely at the type of eye you're treating and adjust your protocol accordingly.

"The data indicate that more patients in the United States and around the world lose vision to undertreatment than to overtreatment," he adds. "So, while I think retina specialists should be concerned about causing atrophy, they should also be concerned about the disease itself, and make sure it's kept under control."

Dr. Rosenfeld agrees. "When patients come to us with exudative disease, not treating them isn't an option," he says. "So they get the injections, and we try to balance the need for reinjection and eliminating macular fluid against our concern about the possibility that overtreatment may exacerbate atrophy."

—CK

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"In fact, we're starting to believe that when the Type 1 lesions go away, you're left with macular atrophy," he says. "Many of my colleagues believe that you need to manage the neovascularization, but you don't want to overtreat it. You want the neovascularization to stay there and just stop leaking. To put it another way, the field has evolved to believe that neovascularization is not the enemy; exudation and hemorrhage are the enemy. The neovascularization may actually be serving a very important function—providing nutritional support for the retinal pigment epithelium. Its appearance may be a normal physiologic response to a pathological condition in which you're losing the choriocapillaris and there's low-grade inflammation in the back of the eye. You need this neovascular complex. You just don't want the exudation."

"We know that patients that look clinically dry can harbor 'non-exudative' Type 1 neovascular tissue, which proliferates beneath the RPE with little clinical or optical coherence tomography evidence of its presence," says Dr. Freund. "There's no fluid and no hemorrhage. Today, however, we have tools like OCTA that let us detect these vessels. The most notable fact is that some of these patients never develop pathologic exudation. The challenge is that we don't know exactly what percentage of those patients will eventually develop vision-threatening fluid and hemorrhage."

Dr. Freund frequently collaborates with Christine A. Curcio, PhD, a professor of ophthalmology at the University of Alabama at Birmingham, who is an expert in the histology of macular degeneration. "We've been performing clinicopathologic correlations for AMD for close to a decade," he says. "If patients from my practice consent, we enroll them in our donor program, and the eye bank recovers the whole globe following their death. Our mission is to correlate the histology with the retinal imaging done in the clinic.

"We're currently studying the eyes of a patient followed for 10 years," he says. "When she first presented, she was in her late 70s, and she'd already lost central vision in one eye from scarring due to neovascular AMD. Surprisingly, even though fluorescein angiography and OCT documented Type 1 neovascularization in her other eye, this eye never developed sight-threating exudation, and retained excellent visual acuity for more than a decade. This unusual disease course was documented with multimodal retinal imaging, which we're now correlating with high-resolution histology. Unexpectedly, it turns out that more than half of her macula was sitting on top of neovascular tissue, and the overlying photoreceptors maintained a healthy structure. The neovascular tissue appears to have maintained nutritional support to the fovea as the patient's native choriocapillaris failed due to aging. So, by the time she died, the health of her outer retina appears to have been maintained by the very same tissue that we all fear is going to cause patients to lose vision—tissue that we often do everything in our power to suppress with our anti-VEGF agents."

Dr. Freund notes that the idea that Type 1 neovascularization could be beneficial isn't new. "This idea was first proposed by Hans E. Grossniklaus, MD and Richard Green, MD," says Dr. Freund. "They hypothesized that Type 1 neovascularization had the potential to recapitulate the normal choriocapillaris, and this concept appears to be supported by the findings in our current donor. In some patients, these non-exudative vessels may actually be helping to preserve the overlying retina. So, the goal may not be to destroy or completely inhibit their growth but to carefully monitor eyes with these vessels so that pathologic exudation can be caught and treated early.

"That means we need to have the right balance," he concludes. "We shouldn't be so aggressive that we destroy a potentially protective mechanism that could benefit our patients in future, but we also shouldn't let it get out of control and damage vision quickly with catastrophic bleeding."

Is Fluid Always a Bad Thing?

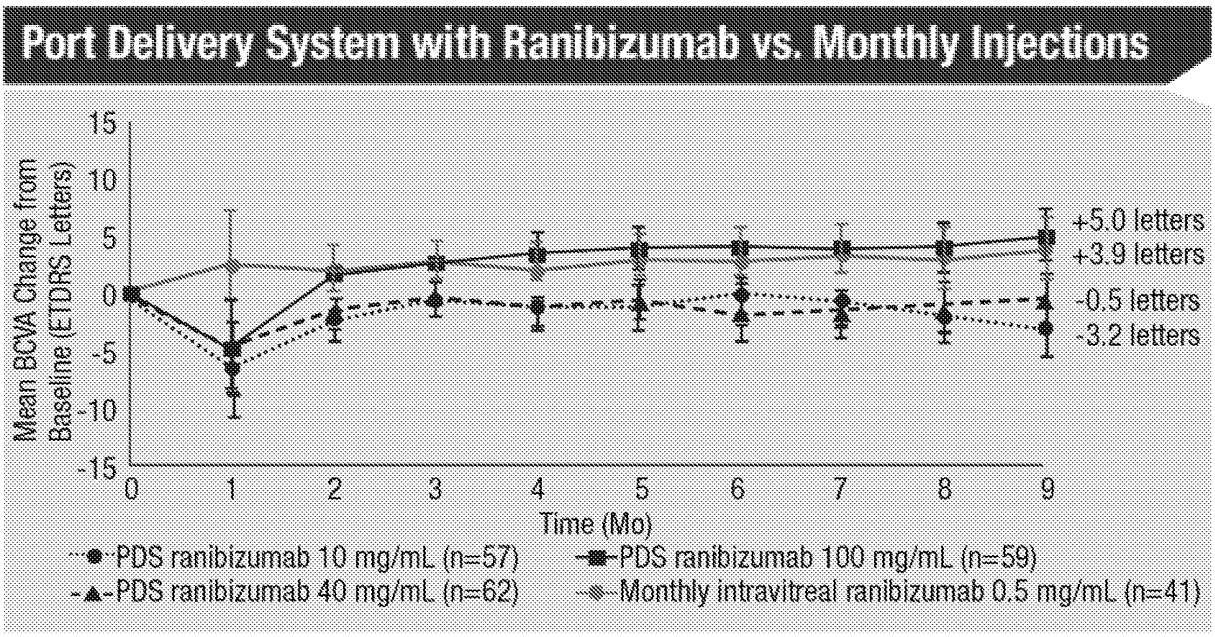
"Studies have shown that eyes treated with intravitreal anti-VEGF therapy which continue to manifest subretinal fluid —eyes that don't go completely dry—may do as well, or even better, than eyes which dry up completely," notes Dr. Freund. "So, a little bit of fluid may not be such a bad thing."

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Dr. Freund refers to the three main types of neovascularization occurring in exudative AMD, and says evidence is mounting that they may warrant different treatment regimens. “I believe retinal specialists should look at the eye’s presenting imaging characteristics, which define a lesion’s subtype,” he says. “Different subtypes seem to respond differently to treatment and call for different levels of monitoring. For example, it’s my strong belief that eyes presenting with Type 1 neovascularization beneath the fovea that continue to show a small amount of subretinal fluid, despite frequent dosing, are more resistant to geographic atrophy than eyes with other neovascular lesion patterns. In these eyes, a little bit of fluid is probably not such a bad thing. The main threat to vision in eyes like this appears to be the occurrence of subretinal hemorrhage if treatment is discontinued.

“In contrast, atrophy is a greater concern in eyes that have intraretinal neovascularization known as a Type 3 neovascularization or RAP (retinal angiomatous proliferation) pattern,” he continues. “Greater susceptibility to macular atrophy in eyes with Type 3 lesions has been observed in several large clinical trials. Patients presenting with Type 3 neovascularization are often older individuals with thin choroids and focal areas of macular hyperpigmentation, both of which increase susceptibility to macular atrophy. For eyes presenting with Type 3 neovascularization, I’ll often first try PRN dosing, for three reasons: lesion quiescence may be long-lasting; recurrences predictably occur at the initial site of activity; and recurrent exudation with Type 3 lesions is rarely accompanied by large hemorrhages.”



Data from the Phase II LADDER clinical trial. All patients were previously treated with and were responsive to anti-VEGF therapy. (Vertical bars=95% confidence intervals.)[4]

Dr. Freund notes that Type 1 and Type 3 are the two most common variants of neovascular AMD. “Type 2 and mixed lesions with a component of Type 2 are less common, but the vessels in these eyes have broken through the RPE to proliferate in the subretinal space,” he says. “You have to be careful with these cases, since recurrent exudation from Type 2 lesions is in direct contact with the vulnerable photoreceptors. In that situation irreversible vision loss can occur quickly.”

Dr. Freund points out that the implication is that different types of lesions should be treated differently. “Don’t simply assume that all neovascular AMD is the same and should be treated with the same regimen,” he says. “Over the short term, these slight variations in regimen may not seem to make a difference, but we often treat patients for the

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rest of their lives. Little differences in how a patient does on one regimen versus another end up making a big difference over five, 10 or 15 years of treatment."

Dr. Freund says that OCT may provide clues to the best treatment protocol. "If you see a shallow, irregular PED with heterogeneous internal contents in a patient with other findings of AMD, there's a high probability that this finding represents neovascular tissue," he says. "If there's no exudation, eyes with this finding should be monitored closely, and the patient should be instructed to use an Amsler grid to monitor for conversion to active wet disease.

"When this type of vascularized pigment epithelial detachment, or Type 1 pattern, is subfoveal and associated with exudation, I use a treat-and-extend regimen of anti-VEGF therapy to control exudation," he continues. "However, my goal is not to flatten the PED itself. I believe that intense treatment has the potential to convert a vascularized PED into avascular fibrotic tissue—tissue that will no longer be able to support the overlying retina. If there's no hemorrhage, I'm not concerned about a little bit of persistent subretinal fluid, as long as it's not increasing over time."

Dr. Freund says that many of his patients have demonstrated the wisdom of this approach. "I have many patients I've been treating with anti-VEGF therapy for 10 or more years, some of whom have had as many as 100 injections," he says. "Many eyes still have close to 20/20 vision."

How Important is OCTA?

"Right now we treat based on exudation—the fluid that's visible on structural OCT," notes Dr. Rosenfeld. "However, people are working hard to see if the information we pick up with OCTA may be able to influence how we treat. Many papers have been published about using OCTA to look at different types of neovascular structures that exist in these eyes with wet AMD, hoping that some of what they find would be predictive of how often we need to treat, and/or the outcome of treatment. The results have been underwhelming, which is probably because the anti-VEGF drugs treat the exudation, not the neovascularization. Thus, changes in the vasculature revealed by OCTA may be less important than the presence of exudation on structural OCT.

"However, OCTA does have a role," he says. "It's a very powerful way to identify eyes with dry macular degeneration that are at higher risk of exudation, so we can follow them differently. With OCTA—in particular SS-OCTA—you can see the neovascularization many months before patients actually develop exudation. It's growing silently. This has been known since the 1970s, when autopsy eyes with dry macular degeneration were shown to harbor neovascularization; it just wasn't leaking or bleeding. Using ICG angiography in the 1990s, retina specialists also showed that this neovascularization existed in their living patients. However, we couldn't routinely screen patients with ICG angiography, so this discovery was largely forgotten until OCTA came along.

"Knowing that this neovascularization is present, via OCTA, is valuable information," he says. "We recently conducted a two-year study involving 227 patients, which is currently in press. We compared eyes with and without this nonexudative neovascularization and found that there's a 14-fold increased risk of exudation over a period of two years when neovascularization is present. So OCTA serves a purpose—just not for monitoring lesions once you start treating. Instead, it can help to identify these lesions before exudation develops.

"For this reason, I use OCTA to check all of my dry AMD patients to see if they have these lesions," he says. "It changes how I manage my patients. If they have the lesions, I usually follow them every two months. If they don't have the lesions, I usually follow them every six months. It also helps me educate patients so they understand what's going on and become partners in their own management."

Dr. Freund says he uses OCTA frequently, but doesn't believe it's essential for diagnosing or treating AMD. "Often, OCT alone is sufficient to diagnose neovascular AMD or to arouse high suspicion that there's a problem," he notes. "In some cases, when there's a characteristic OCT pattern of specific neovascular features, dye angiography may

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not be needed, especially if there's a hemorrhage. I think OCTA is helpful if, like myself, you're interested in identifying the neovascular lesion type to help individualize your treatment algorithm.

"Does individualizing the treatment regimen necessarily lead to better outcomes?" he adds. "I think so, but this remains to be proven. For now, I'd say that OCTA is a valuable research tool. In the future, it may become essential for a clinician managing macular degeneration."

Dr. Rosenfeld believes clinicians will benefit from using OCTA. "I think everyone who practices retinal care needs access to OCTA, whether it's for diabetes or AMD or vein occlusions," he says. "It's a remarkable tool that allows us to see the full extent of the underlying disease, and in certain situations helps us manage it better. OCTA is now our number one tool for diagnosing proliferative diabetic retinopathy. It helps us determine the extent of vascular nonperfusion in diabetics and vein occlusion, and it's particularly useful for identifying the subclinical, nonexudative lesions in dry AMD. Furthermore, patients love it compared with dye-based angiograms. Meanwhile, of course, structural OCT is very useful for managing all of these conditions as well. But with OCTA scans, you get both the structural OCT and the angiographic OCT."

Dr. Rosenfeld points out one big problem with OCTA, however, at least in the United States. "We don't have a unique billing code for OCTA," he explains. "The scan time is about the same as routine OCT, but the time it takes for the physician to interact with the instrument, look at the scans and extract the information by manipulating the segmentation boundaries to get the best possible image isn't currently reimbursed. So, while the equipment is more expensive and it takes more time to evaluate the scans, the reimbursement is the same as for the more typical OCT. Outside the U.S., OCTA has a higher reimbursement, so the technology has become very popular. But in the U.S., it's a difficult product to sell, because doctors want the revenue from dye-based angiography.

"Despite that concern, I believe it's worth using OCTA," he concludes. "With it, you achieve increased patient satisfaction and patient wait time is decreased. You can see more patients, because dye-based angiography takes a long time to perform—and it's risky. So from an economic standpoint I think OCTA wins, even without the unique billing code."

The Syringe Factor

K. Bailey Freund, MD, a retina specialist and a clinical professor of ophthalmology at the New York University School of Medicine, notes that the type of syringe used to deliver the anti-VEGF medication has become a point of consideration. "Unless a syringe is silicone-free, droplets of silicone can end up in the medication," he explains. "Then, if you use syringes that don't have reservoir at the tip, which can trap the silicone as it's pushed down by the plunger, you're more likely to inject some of that silicone into the eye. Compounding pharmacies often use fixed-needle insulin syringes which lack a reservoir at the tip. As a result, some silicone oil often gets into the vitreous.

"Lucentis now offers a pre-filled syringe that's been designed, in part, to minimize this problem," he continues. "Also, with aflibercept, or ranibizumab dispensed in a vial, most retinal specialists use syringes with either a Luer-Lock or Luer-Slip design. These syringes have a dead space at the tip, so when the plunger is pushed all the way down, not all of the fluid in the syringe is injected into the vitreous. However, that type of syringe is frequently not used for bevacizumab, in part because the presence of a dead space requires that more drug be added, which increases the cost.

"The SCORE trial investigators reported this issue with fixed-needle syringes when they were looking at a syringe pre-filled with an Allergan steroid," he says. "Furthermore, a study published in 2017 compared the amount of silicone expressed from syringes of different types.[1] It showed quite convincingly that when you use syringes with fixed needles, you're more likely to see silicone droplets expressed from the needle tip. I'm also a co-author of a recently published editorial in *Retina* which discusses this topic.[2]"

Dr. Freund admits that the number of incidents related to this problem and compounded bevacizumab is very small. "Nevertheless, there are potential issues with a compounded drug," he says. "All of those factors weigh into which drug you choose, even if you think there's not much of a difference between the drugs."

—*CK*

What's Next?

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As effective as the current options are, new drugs and technology in the pipeline have surgeons hoping for even better outcomes—and a reduced injection burden.

• ***Brolucizumab***. This is an investigational anti-VEGF drug from Novartis. "We hope it will provide a greater duration of action," Dr. Rosenfeld explains.

Dr. Freund notes that brolucizumab was compared head to head with aflibercept in a trial. "The trial compared the labeled dosing of Eylea—every four weeks times three, extended to once every eight weeks—to brolucizumab, which is given every four weeks for three injections and then extended to every 12 weeks," he explains. "With brolucizumab, a little more than half of the eyes could be maintained on the 12-week dosing to the final 48-week endpoint. Unfortunately, the trial design makes it hard to do a direct comparison between the drugs because patients treated with aflibercept were never extended beyond eight weeks. However, during the first three months, all the eyes were dosed every four weeks. During that period, there was more improvement in OCT thickness with brolucizumab than with aflibercept. "Part of the reason for this finding is that brolucizumab is a smaller molecule in a more concentrated formulation," he points out. "When you inject the same volume of both drugs, the molar concentration of brolucizumab is approximately 10 times that of aflibercept. That means you're getting a lot more drug in the eye with each injection of brolucizumab."

One reason surgeons look forward to new options is the possibility that they'll help to treat refractory patients. However, Dr. Freund points out that it could be problematic to use brolucizumab for this purpose, at least at the outset, because of the trial design. "Let's say you have a patient that doesn't dry up as much as you hope using one of the other agents," he says. "In the trials of the other drugs there were arms in which patients were treated monthly to the endpoint. Brolucizumab didn't have that, so it seems unlikely that the initial approval will include long-term monthly dosing. The reality is, if a refractory patient can't go for four or five weeks on the other agents, they'll probably have trouble going for eight or 12 weeks with brolucizumab. That may limit how much you can use that drug for these difficult, refractory patients.

"I believe the company is doing other studies that may eventually get a label allowing monthly treatment," he adds, "but at the launch that could be a bit of an issue."

• ***Abicipar pegol***. Another drug being tested is Allergan's abicipar pegol. "The trial design was basically 12-month dosing head-to-head between abicipar pegol and monthly ranibizumab," Dr. Freund explains. "The new drug met its noninferiority endpoint, which is fairly impressive. Patients went to 12 weeks and seemed to do just as well as patients getting ranibizumab every month.

"The issue is that there's been some inflammation with abicipar pegol," he continues. "Initially about 15 percent of patients had inflammation. However, the company subsequently released data from a trial called MAPLE, where a change in the manufacturing of the drug reduced the inflammation rate to 8 or 9 percent, and so far, there's no evidence that the inflammation caused in any of these patients was of much concern. So, you could decide to try abicipar pegol on your patient once. If the patient develops inflammation, you could stop. The risk/benefit ratio would be pretty good: There may be only a 9-percent chance that that the patient will have inflammation, but there's close to a 90-percent chance that the patient will be able to be dosed once every 12 weeks. With brolucizumab you'd only have a 50-50 chance of reaching every 12 weeks. That's one way to look at the data."

• ***Sustained delivery.*** A key way to reduce the injection burden, of course, is via sustained delivery. "No one likes injections," notes Dr. Freund. "But now Genentech has an implantable port that's a refillable reservoir. Theoretically, you'd only have to do two refills a year."

Dr. Rosenfeld says that data from the LADDER study, involving Genentech's Port Delivery System with ranibizumab, was presented at the FLORetina meeting. "It appears to provide some extended benefit for patients," he says. "Less-frequent dosing was needed. In addition, there's a sustained-release tyrosine kinase inhibitor from Graybug Vision (Redwood City, California) that may or may not prove to be a long-term solution for this problem."

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• ***Home monitoring.*** "The key to monitoring those lesions is going to be some kind of home monitoring that's reliable," notes Dr. Rosenfeld. "Our dream device would be an OCT that a patient could use every day, or every other day. That would allow us to identify exudation as soon as it develops. Home OCT monitoring would be useful for following patients who have non-exudative, neovascular lesions that we can identify with OCT angiography, who are at high risk for exudation, as well as patients on a treat-and-extend regimen. If they were testing themselves with home OCT monitoring, we'd know as soon as the fluid develops or recurs.

"Notal Vision has a prototype that's being tested that uses an AI algorithm capable of picking up exudation," he continues. "There are other strategies being developed as well, but the Notal Vision device may become available within a year or two. Patients wouldn't buy the instrument; it would be more of a lease situation. Medicare currently covers the cost of home monitoring, so for a nominal fee, perhaps in addition to Medicare coverage, you'd be able to have your patients monitored at home."

• ***Gene therapy.*** "Two companies have gene therapies that involve injecting a viral vector into the eye," says Dr. Freund. "Adverum Biotechnologies is in a Phase I trial with an intravitreal injection, and Regenxbio will soon be conducting a Phase II trial involving subretinal delivery of gene therapy. The viral vector inserts DNA into cells in the eye, so they start to produce either ranibizumab or aflibercept on their own. Theoretically, you could inject it once and you'd be done. To me that's very exciting." REVIEW

Dr. Freund is a consultant for Allergan, Novartis, Zeiss, OptoVue and Heidelberg Engineering, and receives research support from Genentech Roche. Dr. Rosenfeld has received research funding from and is a consultant for Carl Zeiss Meditec.

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ARTICLE

Intravitreal aflibercept for neovascular age-related macular degeneration in patients aged 90 years or older: 2-year visual acuity outcomes

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Abstract

Purpose The purpose of this study was to investigate the efficacy of intravitreal aflibercept for neovascular age-related macular degeneration (nAMD) in very elderly patients aged 90 years or older at 2 years after treatment initiation.

Methods In this multicentre retrospective data analysis from electronic medical record, consecutive treatment-naive patients with nAMD treated with aflibercept with at least 2 years follow-up were stratified into those aged < 90 years (Group I) and an older cohort aged 90 and over (Group II). We compared the visual acuity (EDTRS letters) outcomes at 4 weekly intervals between the two groups over a 2-year period.

Results The mean visual acuity of Group I at presentation was 56.3 ETDRS letters versus 52.8 letters in Group II. Maximal visual acuity was achieved in both the groups by 6 months after initiating treatment (4.7 vs. 4.0 letters gain). By 2 years, the mean visual acuity of the older cohort fell marginally below their baseline visual acuity (0.8 letter loss), while Group I presented +2.1 letters gain. The number of injections given and the retention rate of the older cohort were no different to the rest of the patients.

Conclusions Very old patients with nAMD benefited from aflibercept, but not to the same degree as the younger patients. The study showed that, on an average, the very elderly patients were able to adhere to the intensive anti-VEGF treatment regimens.

Introduction

Age-related macular degeneration (AMD) is broadly classified into early and late forms. Late AMD is the leading cause of visual loss in the elderly. AMD is a perfect example of an aging-dependent condition, where the prevalence of the disease increases monotonically with age [1]. The prevalence of late AMD increases from 1% at 70 years to as high as 12% in patients over 80 years [2–4]. This condition is an outcome of a multitude of accumulating degenerative changes in the various layers of the outer retina and the choroid. Although there have been many well-conducted epidemiological studies on AMD, the proportions of patients aged 90 years or older in these cohorts were small. Therefore, there is a paucity of information on AMD in the very elderly patients.

Late AMD is further classified into "dry" and "wet or neovascular" (nAMD). The former is responsible for the majority of cases, but there are no treatment options available for this condition as yet. Only 10–15% of patients exhibit nAMD and these patients present with sudden decrease in vision, and if left untreated, this condition results in profound irreversible visual impairment [5, 6]. AMD is a bilateral disease and the risk of developing nAMD in the fellow eye of patients with unilateral AMD

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increases by 10% per year from the date of diagnosis, albeit this is dependent on features of the eye and other risk factors [7]. Furthermore, if a patient develops nAMD in one eye at the age of 80, the risk of nAMD in the fellow eye is 48% at 10 years [6]. Therefore, we would expect most patients with nAMD at 90 years to have bilateral nAMD. Determining the outcome of treatment in this cohort, compared to those in the younger age group, has implications, as it estimates the burden of this disease in this age group that is also at risk of other aging-dependent co-morbidities. Studying the treatment outcomes in this group will also provide us with better estimates of age-dependent outcomes of treatments for health economic evaluation. Ultimately, this has significant implications for future service provision, and treatment planning in the very elderly.

Intravitreal injections of inhibitors of vascular endothelial growth factor (VEGF) have been the mainstay of treatment for nAMD for over a decade [8–12] The three anti-VEGF agents commonly used for this condition include ranibizumab, bevacizumab and aflibercept. The treatment regimens are intensive with monthly intravitreal injections for 3 months, followed by varying re-treatment regimens depending on the anti-VEGF agent. On an average, a patient requires 7–8 injections in the first year and six injections from the second year. A comprehensive assessment of baseline predictive factors for visual outcomes at month 12 in Comparison of Age-related macular degeneration Treatment Trials (CATT) demonstrated that older age was associated with poor outcomes in both ranibizumab and bevacizumab groups, while the exact cause for this observation remains unknown [10]. Aflibercept is the newest FDA-approved anti-VEGF agent against nAMD, which binds tightly to VEGF-A, having a longer half-life than both ranibizumab and bevacizumab, a property which can be exploited by ophthalmologists to allow greater intervals between intravitreal treatments [12] The VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD studies (VIEW 1 and VIEW 2) showed that intravitreal aflibercept given bimonthly after 3 initial monthly doses had comparable improvement in visual acuity as monthly ranibizumab in treatment-naive patients with nAMD [12]

To our knowledge, the specific effect of older age on the efficacy of aflibercept in nAMD has not been addressed in the scientific literature, while older age has been shown to adversely affect the outcomes in patients treated with both bevacizumab and ranibizumab [10, 11] In addition, since it is difficult to study the outcomes in an elderly population due to several issues, including follow-up retention, large study samples are needed in order to reach reliable conclusions. Consequently, analysis of a large database system seems to be a good option. In fact, the use of electronic databases to estimate the outcomes in retinal disorders is becoming increasingly common, and they have been used to provide estimates for national and regional population outcomes [13, 14]. We have recently utilized a national dataset from 16 centres in the United Kingdom (UK) to report that the "real world" clinical outcomes of aflibercept in nAMD are similar to that of the randomized control trials of aflibercept in AMD [13] Therefore, this dataset is ideal to examine the influence of advanced age on visual outcomes in patients with nAMD treated with aflibercept. In this study, we compared aflibercept the outcomes in very elderly patients with nAMD (≥90 years) with the rest of the cohort treated with aflibercept for nAMD to evaluate the treatment's outcomes in the very old population.

Materials and methods

In this retrospective study, anonymised data was extracted from 16 UK National Health Service (NHS) hospitals forall treatment-naive eyes with nAMD initiated on aflibercept between March 2013 and April 2015, to ensure a minimum of 24-month follow-up. All the data were recorded using a single electronic medical record (EMR) system (Medisoft Ophthalmology; Medisoft Limited, Leeds, UK), which mandated the collection of a standardized dataset throughout the nAMD care pathway. Written approval for anonymised data extraction was received from each participating hospital. Ethical approval was not required, since a study of this nature is considered as an audit or service evaluation. The study was conducted in accordance with the tenets of Helsinki Declaration and the UK's Data Protection Act.

Sites were selected based on the confirmation that they used aflibercept to treat nAMD according to the VIEW protocol, consisting of 3-monthly intravitreal aflibercept injections (loading phase), followed by repeated bimonthly injections thereafter during theentire year 1 . All included centers were using a treat and extend approach after year 1. To determine the influence of age on visual acuity (VA) outcomes, patients were divided into two groups: Group I consisted of a younger cohort aged 0–89 years, while Group II consisted of very elderly patients aged 90 years and over.

All patients underwent optical coherence tomography (OCT) and fluorescein angiography to confirm the diagnosis of nAMD, while indocyanine green angiography was used at the discretion of the physician when needed. Eyes with polypoidal choroidal vasculopathy, choroidal neovascularization of other cause than nAMD, other co-morbidities that may confound visual outcomes and those treated previously with anti-VEGF were excluded. At each visit, visual acuity, injection history and follow-up date were recorded and entered into the EMR system by all staff members as part of routine clinical care. Potential operative and postoperative ocular and systemic complications were also documented at each visit.

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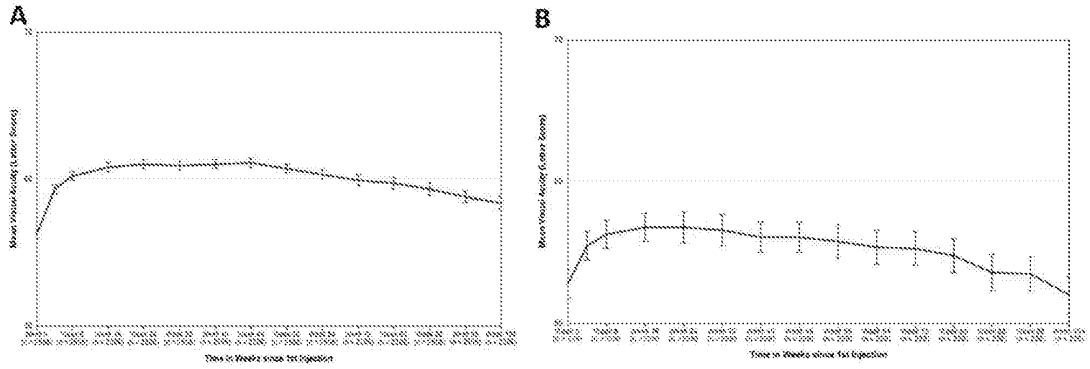


Fig. 1 Mean change in visual acuity over time in patients aged <90 years (**a**) and ≥90 years (**b**)

Visual acuity and missing value imputation

Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity letter scores at 2 meters were recorded at each visit at all sites, and represented the primary outcome of the study. In cases when alternatives measures were used conversion to ETDRS letters was performed. The visual acuity at each clinical visit was used for the analysis. When visual acuity recorded was counting figures, hand movements, light perception or no light perception, zero ETDRS letter score was ascribed.

Since in real-world studies, patients tend not to attend at the precisely intended intervals, the visual acuity of all attendees at 4-week intervals were utilized for the analysis over a 2-year period, having ensured that the sample size in each month reflected all patients who were being followed up continually. If there were gaps in the visual acuity data, they were imputed using the mean of the observations before and after the missing period. No observations were carried forward beyond the last recorded VA value, because loss to follow-up is more common in clinical practice than in proper clinical trials.

Results

Data were extracted from 16 NHS centers for 2734 treatment-naive eyes initiating aflibercept treatment for nAMD between March 2013 and April 2015, to allow a minimum follow-up of 2 years. Group I (aged < 90 years) comprised 2506 eyes and Group II (aged ≥ 90 years) included 228 (8.3%) eyes.

Mean change in visual acuity at 2 years

The mean change in VA over time for both the groups is shown in Fig. 1. In patients aged < 90 years, the mean VA at baseline was 56.3 ETDRS letters (standard error, 0.3 letter), improving significantly to 60.9 ETDRS letter at year 1 (standard error, 0.3 letter; + 4.6 letters gain) and to 58.4 ETDRS letter at year 2 (standard error, 0.4 letter; + 2.1 letters gain), compared to baseline. In patients aged ≥ 90 years, the VA at baseline was 52.8 ETDRS letters (standard error, 1 letter) and improved to 55.9 ETDRS letters at year 1 (standard error, 1.2 letter; + 3.1 letters gain), but dropped to 52 ETDRS letters at year 2 (standard error, 1.3 letter; −0.8 letters loss), compared to baseline. The maximum change in VA was achieved at 6 months for both the groups (+4.7 and +4.0 letters gain respectively).

First eye involvement

If analysis was restricted to first-treated eyes (eyes with normal vision in the other eye), in Group I the mean baseline VA for first-treated eyes was 55.2 ETDRS letters (standard error, 0.4 letter), which increased to 60.6 letters at year 1 (standard error, 0.4 letter; +5.4 letters gain) and to 58.1 letters (standard error, 0.5 letter; +5 letters gain) at year 2. In Group II, the mean baseline VA in first-treated eyes was 50.0 ETDRS letters (standard error, 1.2 letter), increasing to 52.4 letters at year 1 (standard error, 1.5 letter; +2.4 letters gain), but dropping to 48.8 letters at year 2 (standard error, 1.7 letter; −1.2 letters loss).

Second eye involvement

For second-treated eye (eyes with visual loss in other eye) in Group I, the mean baseline VA was 63.5 ETDRS letters (standard error, 0.7 letter), increasing to 65.1 letters at year 1 (standard error, 0.8 letter; +1.6 letters gain) and to 63.4 letters at year 2 (standard error, 0.8 letter; −0.1 letter loss). In Group II, the mean baseline VA for second-treated eye was 59.2 ETDRS letters (standard error, 2.4 letter), which increased to 63.0 letters at year 1 (standard error, 2.2 letter;

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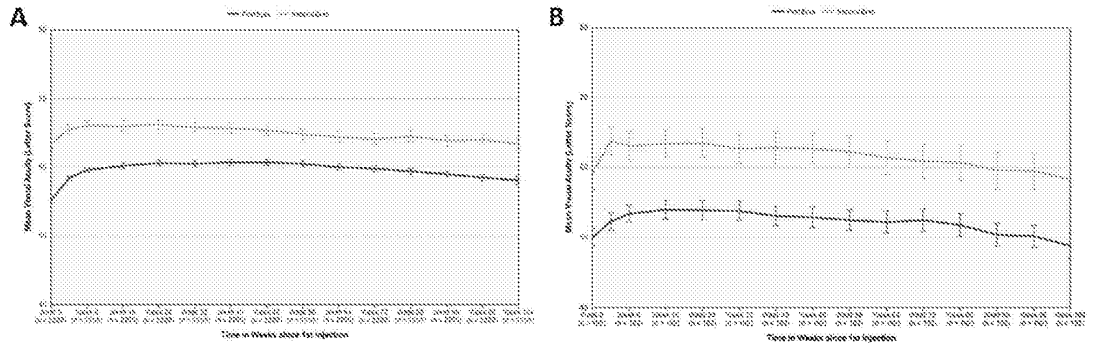


Fig. 2 Mean change in visual acuity over time regarding first-treated and second-treated eyes in patients aged <90 years (**a**) and ≥90 years (**b**)

+3.8 letters gain) and dropped to 58.3 letters at year 2 (standard error, 2.5 letter; −0.9 letter loss), as it is shown in Fig. 2.

Patients presenting poor visual acuity

There were 218 eyes (13.0%) in Group I, compared to 24 eyes (10.5%) in Group 2 that presented with VA < 35 ETDRS letters. For both the groups, the largest VA gains were achieved in eyes that started with the worst vision. In patients aged < 90 years, eyes with baseline VA < 35 ETDRS letters gained a mean of 11.6 letters (standard error, 1.1 letter) and 9.2 letters (standard error, 1.2 letter) at year 1 and 2 respectively. A similar trend was observed in patients aged ≥ 90 years with eyes commencing aflibercept treatment, with the worst VA attaining the largest VA gains, but to a lesser degree. Specifically, patients in Group II with baseline VA < 35 ETDRS letters gained a mean of 7.4 letters (standard error, 1.9 letter) and 3.9 letters (standard error, 3.4 letter) at year 1 and 2, respectively.

Patients presenting good vision

There were 397 patients (15.8%) in Group I, compared to 24 (10.5%) patients in Group II who presented VA > 70 ETDRS letters. In Group I, there was a mean decline of 0.6 letters (standard error, 0.4 letter) and 3.5 letters (standard error, 0.6 letter) at year 1 and 2, respectively, as it is depicted in Fig. 3a. In Group II, patients with baseline VA > 70 ETDRS letters (24 eyes) experienced a significant drop in VA of 3.8 letters at 1 year (standard error, 1.7 letter) and 13.3 letters at year 2 (standard error, 3.4 letter), as shown in Fig. 3b.

Gains in visual acuity

In Group I, the proportions of the 2147 eyes (85.7%) with follow-up at year 1 that gained 5, 10 and 15 letters were 52.3%, 35.2% and 20.6%, respectively. At year 2, the proportions of the 2102 eyes (83.9%) with a gain of 5, 10 and 15 letters were 46.7%, 31.5% and 20.7%, respectively. In patients aged ≥ 90 years, the proportions of 186 eyes (81.6%) with follow-up at 1 year that gained 5, 10 and 15 letters were 49.5%, 32.8% and 22.6%, respectively. At year 2, the proportions of the 192 eyes (84.2%) gaining 5, 10 and 15 letters were 37.5%, 27.6% and 17.7%, respectively (Fig. 4).

Losers in visual acuity

Over the first year, the proportions of those losing 5, 10 and 15 letters in Group I were 18.8%, 10% and 5.8%, respectively and at 2 years, 26.3%, 18.3% and 13% lost 5, 10 and 15 letters. In Group II, the proportions losing 5, 10 and 15 letters were 26%, 14% and 7%, respectively at year 1 and 37%, 24% and 16% of eyes in year 2, respectively.

Discussion

This study evaluated aflibercept outcomes in nAMD in the largest series of patients aged 90 years or over. The principal message of this study is that intravitreal aflibercept is effective in the very elderly population with nAMD and they are able to adhere to rigorous anti-VEGF treatment regimens.

There are half a million people aged 90 years or older in the UK. Owen et al reported ~40,000 new nAMD cases a year in the UK, with an estimated 7700 people beingin this age group (19%) [4]. This study shows that the oldest people constituted 8% of the new cases presented, and were treated with aflibercept over 2 years. Therefore, approximately half the expected number of the very elderly patients with new onset nAMD were present for the treatment. Considering those who presented for diagnosis and

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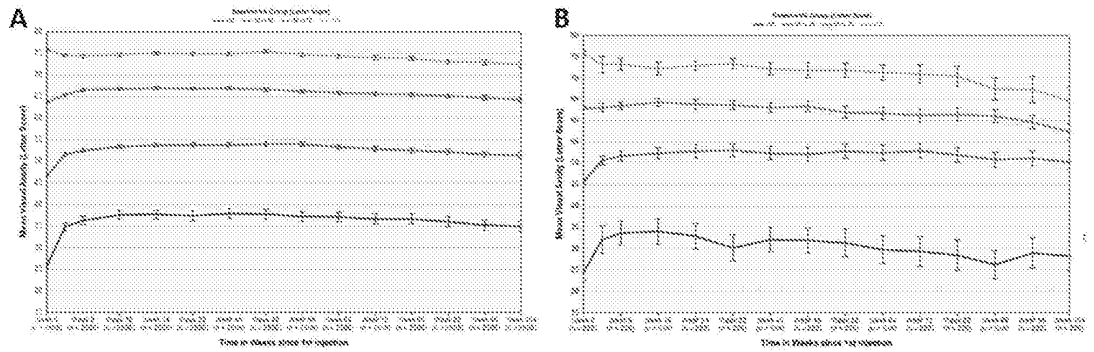


Fig. 3 Mean change in visual acuity over time based on baseline visual acuity groups in patients aged <90 years (**a**) and ≥90 years (**b**)

treatment, their baseline VA was similar to the rest of the cohort. Therefore, those were the patients who were able to access the NHS benefit from the treatment received for nAMD.

The proportion of patients with final VA records at 2 years was also similar between the two the groups, suggesting that the drop out rate was not higher in the very old patients. The study is limited to 2-year follow-up. Longer follow-up of these patients will provide insight into any deviations between the two cohorts on clinic attendance with time. The outcome measured in various ways show that very elderly patients with nAMD benefit from aflibercept therapy. However, it is more challenging for the patients aged 90 years or above to achieve the driving vision, defined as more than 70 letters in the treated eyes. In addition, the older patients who presented good vision (>70 letters) showed a larger decline in VA over the 2 years, compared to the rest of the cohort. These outcomes in this age group may contribute to the higher risk of falls and fractures, need for nursing home support and mortality.

Interestingly, we also observed that 80% of patients presented first eye involvement in the 90 years and above group. We postulate that this group may indeed represent a unique "special" group that has avoided conversion to nAMD, probably having a lower predisposition for the development of nAMD. It would be useful to interrogate this group of patients further in terms of both functional and structural changes in the retina to study the disease mechanism.

The VA outcomes of the present study were not as favorable as the clinical trials outcomes, but were similar to the other real-life studies [13, 15, 16] The mean gain in VA in our study in patients aged < 90 years was +4.6 and +2.1 letters at year 1 and 2, respectively. In patients aged ≥ 90 years, the mean gain in VA at year 1 was +3.1 letters, while patients lost 0.8 letters at year 2. The integrated analysis of VIEW 1 and VIEW 2 studies showed 8.4 letters gain at year 1 (7.9 letters for VIEW 1 and 8.9 letters for VIEW 2 study [12], whereas Talks et al found 5.1 letters gain at year 1 in patients with nAMD treated with intravitreal aflibercept in real-life conditions, based on the VIEW protocol [13]. Additionally, previous audits using PRN ranibizumab in clinical practice reported VA gains of 2–3.8 letters at year 1 [15, 16]. It is worthy to note that reduced VA in the older group may represent more prevalent concurrent atrophic changes than in the younger group. In addition, the aged outer retinal tissue may be less responsive to anti-VEGF therapy.

The discrepancy in the VA outcomes between clinical trials and real-life studies may be explained by the fact that in the real-world, data measurement techniques may differ. Specifically, VA is usually measured with the patients' habitual correction and not with the subjective refraction at each visit as in clinical trials. Although this may underestimate the actual changes in vision, it may reflect better patients' vision experience [13]. Moreover, patients in clinical trials are strictly selected and may have different characteristics than those of daily practice, while treatment is administered within tight timelines. It is also worthy to note that this older patient cohort is often not included in the clinical trials, even though these diseases are aging dependent. They represented 8% of this cohort. Therefore, this is an example where the answers to a research question are better derived from real-life datasets than clinical trials.

Another interesting observation of our study was that maximal VA gain was obtained at month 6 after initiation of treatment, irrespective of the age. In addition, the initial VA gain achieved after the 3-monthly loading phase was maintained at the end of year 1 in both the groups and by year 2 in patients aged < 90 years. In contrast, in the very elderly patients, the rate of decline in VA was more pronounced after year 1, with patients presenting loss of 0.8 letters at year 2 and falling below the baseline VA. However, the outcome is still better than the natural history of the disease. Although treatment after 6 months did not result in further VA gains in either group, continuous treatment may halt further visual deteriorations in both the groups.

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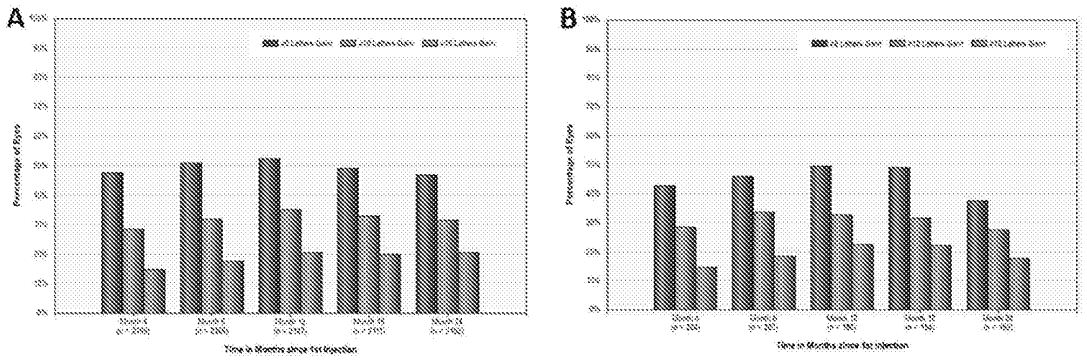


Fig. 4 Percentage of patients gaining at least 5, 10 and 15 letters in patients aged <90 years (**a**) and ≥90 years (**b**)

In conclusion, our study showed that very old patients with nAMD benefited from aflibercept therapy, but not to the same degree as the younger patients. The reason why elderly patients do not achieve the same outcomes as their younger counterparts despite adhering to the treatment regimen is unclear. An understanding of the morphological differences in patients with nAMD across different age profiles should shed more light on the disease mechanisms. The information provided in this study may also be of value to health economists for incorporation in future cost-effectiveness models.

Summary

What was known before

- Intravitreal aflibercept is safe and effective for the treatment of age-related macular degeneration.
- The so-far studies regarding the effectiveness of aflibercept in patients over 90 years are scarce.

What this study adds

- Very old patients with nAMD benefited from aflibercept, but not to the same degree as younger the patients.
- The study showed that on an average, the very elderly patients are able to adhere to the intensive anti-VEGF treatment regimens.

UK Aflibercept Users Group A. J. Lotery, F. Ghanchi, N. Patel, C. Bailey, S. Mahmood, A. Lobo, B. Paul, Q. Kashif, C. Santiago, G. Walters, M. Tahir, B. Mushtaq, K. Ahmed, M. McKibbin, S. Sivaprasad, and J. Talks

Compliance with ethical standards

Conflict of interest Irini Chatziralli, James Talks and Sobha Sivaprasad have received travel grants, research grants and speaker fees, and attended advisory board meetings of Bayer, Allergan and Novartis. The remaining authors declare that they have no conflict of interest.

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Ziv-aflibercept: A novel angiogenesis inhibitor for the treatment of metastatic colorectal cancer

CLEMENT CHUNG AND NISHA PHERWANI

Purpose. The pharmacology, pharmacokinetics, clinical efficacy, safety, and administration of ziv-aflibercept in combination therapy for metastatic colorectal cancer (mCRC) are reviewed.

Summary. Ziv-aflibercept (Zaltrap, Regeneron Pharmaceuticals and sanofi-aventis) is a novel recombinant fusion protein that targets the angiogenesis signaling pathway of tumor cells by blocking vascular endothelial growth factor (VEGF) receptors that play a key role in tumor growth and metastasis; it is a more potent VEGF blocker than bevacizumab. Ziv-aflibercept is approved by the Food and Drug Administration for use in combination with fluorouracil, irinotecan, and leucovorin (the FOLFIRI regimen) for second-line treatment of patients with mCRC who have disease progression during first-line oxaliplatin-based chemotherapy. A Phase III trial demonstrated that relative to FOLFIRI therapy alone, the use of ziv-aflibercept was associated with significantly improved patient response, overall survival, and progression-free survival in patients with good performance status at baseline, including some who had received prior bevacizumab therapy. The most common grade 3 or 4 adverse effects associated with ziv-aflibercept use in clinical studies were neutropenia, hypertension, and diarrhea; the U.S. product labeling warns of potential hemorrhage and other treatment-related risks.

Conclusion. Current clinical data are insufficient to directly compare ziv-aflibercept and bevacizumab when used with standard combination chemotherapy as first- or second-line regimens for mCRC. The role of ziv-aflibercept is currently limited to the second-line setting in combination with irinotecan-based regimens in mCRC patients who have not received irinotecan previously. The role of ziv-aflibercept in chemotherapy for other tumor types is yet to be determined.

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Cancers of the colon and rectum (collectively, colorectal cancer) comprise the third most common tumor type worldwide, with the former being more prevalent than the latter.[1] Colorectal cancer is the third most common form of cancer in the United States and the second most common cause of cancer-related deaths[2]; an estimated 143,460 new cases and 51,690 deaths occurred in 2012.[3] It is estimated that 20% of patients with colorectal cancer have metastatic disease at the time of diagnosis. Despite optimal treatment with adequate surgery and adjuvant chemotherapy, 30–50% of patients with primary colon cancer relapse and die from metastatic disease.[4,5] For most patients with metastatic colorectal cancer (mCRC), treatment remains palliative and the five-year survival rate is disappointing, at approximately 10%.[4]

First-line treatment of mCRC typically consists of fluorouracil and leucovorin (abbreviated FO, for folinic acid, in regimen acronyms) combined with either an oxaliplatin-based regimen (i.e., FOLFOX chemotherapy) or an irinotecan-based regimen (i.e., FOLFIRI), with crossover to the alternative regimen for second-line treatment (i.e., first-line FOLFOX followed by second-line FOLFIRI chemotherapy or vice versa).[6] Various trials have demonstrated no significant difference between FOLFOX and FOLFIRI chemotherapy in terms of the average response rate (54% and 56%, respectively), progression-free survival (PFS) (8.0 months versus 8.5 months), and overall survival (OS) (20.6 months versus 21.5 months) in patients with mCRC.[7-9] However,

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the trial results indicated differences in the toxicity profiles of the two regimens, with relatively more frequent grade 3 or 4 mucositis, nausea and vomiting, and grade 2 alopecia with FOLFIRI chemotherapy and more frequent grade 3 or 4 neutropenia and neurotoxicity with FOLFOX chemotherapy.[10,11]

In the last decade, the introduction of targeted biological therapy has transformed the paradigm for treatment of mCRC. Antiangiogenesis agents, such as bevacizumab,[12] and epidermal growth factor receptor antagonists, such as cetuximab,[13] have been added to the standard combination chemotherapy (FOLFOX or FOLFIRI), with improved survival outcomes reported in patients with mCRC. Angiogenesis, the process by which new blood and lymphatic vessels are formed, is required to support growth in the embryo and young animals, as well as to allow tissue repair and remodeling in adults.[14] For tumors, as with normal tissues, oxygen and nutrients are obtained and waste products are removed via the vasculature. Although tumors can grow in part by co-opting existing host vessels,[15] most tumors induce new vessel formation (neovascularization), suggesting that angiogenesis is a hallmark of tumor growth and function throughout the tumor's life cycle. The predominant growth factor, or proangiogenic factor, of the angiogenesis pathway is vascular endothelial growth factor (VEGF).[16,17] Its continuous expression by the tumor makes VEGF a rational target for cancer therapy.[17]

This article focuses on the clinical review of ziv-aflibercept (ziv″ - a flib′ er sept; Zaltrap, Regeneron Pharmaceuticals and sanofi-aventis), a novel fully humanized monoclonal antibody with antiangiogenic activity recently approved by the Food and Drug Administration (FDA) for use in combination with second-line FOLFIRI chemotherapy for patients with mCRC who have not responded to an oxaliplatin-containing first-line regimen.

Pharmacology

The mammalian VEGF family[14,18] consists of six distinct glycoproteins, or growth factors: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF). These growth factors are ligands that bind to VEGF receptors that mediate tumor angiogenesis. Among these, VEGF-A (or simply VEGF) is considered the principal driving force in tumor angiogenesis.[18] Each ligand has specific affinity for different receptors.

There are three VEGF receptors: VEGFR-1, VEGFR-2, and VEGFR-3. All of these VEGF receptors are characterized by seven immunoglobulin (Ig)-like domains in the extracellular region, a single transmembrane domain, and an intracellular tyrosine kinase domain.[19] These VEGF receptors are high-affinity (denoted by low dissociation constant) receptors, with tyrosine kinase activity expressed in normal and tumor vasculature (i.e., vascular endothelial cells).[18,20] The major receptor for VEGF-A is VEGFR-2 (flk1/KDR). Although VEGF-A binds to VEGFR-1 with 10 times higher affinity than does VEGFR-2, the higher tyrosine kinase activity of VEGFR-2 makes it the most important effector in VEGF signaling.[18,20] Of note, PlGF, which binds to VEGFR-1 but not VEGFR-2, also contributes to tumor angiogenesis.[19]

Ziv-aflibercept, a fully humanized soluble recombinant fusion protein, is created by fusing extracellular Ig domain 2 of VEGFR-1 and extracellular Ig domain 3 of VEGFR-2 to the Fc (constant) region of human IgG1, resulting in the formation of a more potent and high-affinity VEGF blocker than bevacizumab.[19] This fusion protein has been demonstrated to have pharmacologically improved activity over bevacizumab (Table 1).[21-23] Since this fusion protein blocks the angiogenesis pathway by binding to all isoforms of VEGF-A, VEGF-B, and PlGF, it is also known as a "VEGF trap" or a composite decoy receptor (due to the fusion of both VEGFR-1 and VEGFR-2 to IgG1).[21] By binding to these endogenous ligands, ziv-aflibercept inhibits the binding and activation of their cognate receptors, resulting in inhibition of downstream signaling events that mediate neovascularization and vascular permeability. This process results in maintenance of a more functional and normal vasculature,[18,19] inhibition of tumor growth and tumor metastasis, and potentially improved drug delivery (due to "normalization" of tumor vasculature that enables tumor cells to become more sensitive to cytotoxic chemotherapy).[24]

Table 1.
Key Differences in Biological Properties of Ziv-aflibercept and Bevacizumab[21-23,a]

Property	Ziv-aflibercept	Bevacizumab
Binding target(s)	VEGF-A, VEGF-B, PlGF	VEGF-A
Equilibrium dissociation constant,[b] pM	0.49	58
Elimination half-life, days	5–6	20

[a]VEGF = vascular endothelial growth factor, PlGF = placental growth factor.
[b]A measure of binding affinity, with smaller values indicating stronger binding.

Pharmacokinetics and pharmacodynamics

Ziv-aflibercept binds 1:1 to endogenous VEGF to form stable inert complexes, which appear in the circulation at a maximum level within 24–48 hours of treatment.[25]

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These complexes are retained in the systemic circulation. Clearance occurs via a receptor- or pinocyte-mediated pathway that results in proteolysis.

Pharmacokinetic studies of ziv-aflibercept involved the measurement of plasma concentrations of free and VEGF-bound ziv-aflibercept.[26-28] The level of VEGF-bound ziv-aflibercept indicates the amount of endogenous VEGF produced in normal and tumor tissues, whereas free ziv-aflibercept is available for binding with newly secreted VEGF. Free ziv-aflibercept concentrations exhibit linear kinetics at a dose range of 2–9 mg/kg, with steady-state concentrations of free ziv-aflibercept reached by the second dose. Maximum VEGF-bound ziv-aflibercept levels are reached at doses of 2 mg/kg i.v. or greater, indicating complete blockade of the ligand. In pharmacokinetic studies, the half-life of ziv-aflibercept increased (from 1.7 to 5.1 days) as doses were increased from 0.3 to 7.0 mg/kg i.v. Clearance was stable at doses in the range of 2–7 mg/kg. There was no accumulation of free ziv-aflibercept between treatment cycles.

Some studies also demonstrated that the biological effects of ziv-aflibercept correlated closely with free ziv-aflibercept levels in excess of VEGF-bound ziv-aflibercept levels.[26-29] The mean maximum observed plasma concentration of free ziv-aflibercept, at doses ranging from 0.3 to 7.0 mg/kg (0.3 mg/kg is about a tenth of the biologically effective dose in humans), varied from 4 to 159 μg/mL.[29] Following the administration of ziv-aflibercept at 2- and 3-mg/kg doses, free drug concentrations were similar to VEGF-bound ziv-aflibercept concentrations. With doses of ≥4 mg/kg, free ziv-aflibercept concentrations were in excess of bound ziv-aflibercept concentrations throughout the dosing intervals in most patients. Thus, a 4-mg/kg i.v. dose provides a sufficient ziv-aflibercept concentration to block endogenous production of VEGF.

Van Cutsem and colleagues[26] showed that clearance and concentrations of free and bound ziv-aflibercept at steady state in patients treated with irinotecan-based combination chemotherapy were comparable to those observed with ziv-aflibercept monotherapy. No clinically significant pharmacokinetic drug–drug interactions were found between ziv-aflibercept, irinotecan, and SN-38 the active metabolite of irinotecan).

Clinical trials

Phase I research. The safety and dose determination of single-agent ziv-aflibercept therapy were explored in a dose-escalation trial conducted by Lockhart and colleagues[29] that included 7 patients with mCRC among a total of 47 patients with refractory solid tumors. Seven dose levels were evaluated (0.3, 1.0, 2.0, 3.0, 4.0, 5.0, and 7.0 mg/kg); 3, 7, 6, 7, 7, 4, and 13 patients were treated at each respective dose level. The investigators also evaluated the safety and maximum tolerable dose (MTD) of ziv-aflibercept at doses ranging from 0.3 to 7.0 mg/kg i.v. every two weeks. The MTD was defined as the highest dose at which 2 of 3–6 treated patients experienced a dose-limiting toxicity (DLT): uncontrolled hypertension, grade 3 or 4 proteinuria, febrile neutropenia, grade 4 neutropenia, grade 3 or 4 thrombocytopenia, and any toxicity of any grade that led to drug discontinuation. DLTs were evaluated during each dose-escalation phase. Tumor response was evaluated after every two cycles (or four treatments per eight-week interval) according to the RECIST (Response Evaluation Criteria In Solid Tumors, version 1.0) scheme. Patients could elect to continue treatment until disease progression or intolerable toxicity or until they chose to withdraw consent.

Results showed that single-agent ziv-aflibercept was generally well tolerated, with a rate of partial response of 6% and a rate of stable disease for greater than one year of 4%. The types of treatment-related toxicities observed (e.g., fatigue, hypertension, proteinuria) were consistent with those reported in other trials evaluating antiangiogenic therapies.[30-34] The median times to the onset of hypertension or proteinuria were 3.5 days (range, 1–21 days) and 15 days (range, 14–16 days), respectively. These adverse events were reversible on drug discontinuation or initiation of supportive care. Based on these observations and observed increases in the frequency and severity of adverse events at doses of ≥4 mg/kg, single-agent ziv-aflibercept 4 mg/kg i.v. every two weeks was determined to be the recommended dosage for use in Phase II trials.

In another study, investigators evaluated the safety, efficacy, DLT profile, and recommended dose of ziv-aflibercept in combination with irinotecan-based chemotherapy for second-line treatment of Japanese patients with mCRC, 10 (63%) of whom had received prior bevacizumab therapy.[28,35] Ziv-aflibercept was administered at two dosage levels (2 and 4 mg/kg i.v. every two weeks, administered to 3 and 13 patients, respectively). The median numbers of cycles administered were 6 (range, 3–9) and 10 (range, 1–23), and the total numbers of cycles of the 2- and 4-mg/kg doses were 18 and 131, respectively. No DLTs were observed at either dosing level. The response rate and PFS for patients receiving the 4-mg/kg i.v. dose were 8% and 7.6 months, respectively. At both dosing levels, the most common adverse events (occurring in ≥40% of patients) included bone marrow suppression (e.g., neutropenia) and gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, stomatitis, decreased appetite); these toxicities are known to be associated with FOLFIRI regimens. In addition, toxicities known to be associated with

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the antiangiogenesis therapy, such as hypertension and epistaxis, were also frequently observed (rates of ≥40%) with use of the 4-mg/kg dose. The most common grade 3 or 4 adverse events were neutropenia and hypertension. This study established the recommended dose and schedule of ziv-aflibercept (i.e., 4 mg/kg i.v. every two weeks) to be used in combination with irinotecan-based chemotherapy for mCRC.

Of note, ziv-aflibercept has been evaluated extensively for the treatment of patients with other advanced solid tumors.[30-34] Most of the patients involved had received prior chemotherapy regimens, either as first- or higher-line therapies. Ziv-aflibercept was found to have very modest clinical activity as a monotherapy; however, when it was used as a component of combination therapies, partial-response rates ranged from 4% to 18%.

Phase II research. Tang and colleagues[36] conducted a multicenter open-label Phase II trial in 75 heavily treated mCRC patients with good performance status (i.e., Eastern Cooperative Oncology Group [ECOG] performance status of 2 or less). Patients had received a median of two prior regimens and were stratified as having received (n = 51) or not received (n = 24) prior bevacizumab therapy. The primary endpoints were response rate and PFS. After a median of 4 treatment cycles (range, 1–16 cycles) of single-agent ziv-aflibercept therapy (4 mg/kg i.v. every 2 weeks), median PFS was 2.4 months among patients who had received prior bevacizumab therapy and 2.0 months in the bevacizumab-naive group. The efficacy of ziv-aflibercept did not seem to be affected by prior bevacizumab therapy (duration not specified). In the prior-bevacizumab cohort, 1 patient had a partial response and 6 patients (12%) had stable disease for greater than 16 weeks. In the bevacizumab-naive cohort, the best response was stable disease for greater than 16 weeks in 5 of 24 patients (21%). Overall, the most common adverse events of any grade were fatigue (68%), hypertension (51%), proteinuria (49%), headache (42%), voice alteration (31%), anorexia (24%), and joint pain (18%). Single-agent ziv-aflibercept appeared to be tolerated and to have modest antitumor activity in pretreated patients with mCRC, including those who had received prior bevacizumab therapy.

A notable limitation of the trial of Tang et al.[36] was that the activity of ziv-aflibercept against mCRC has never been directly compared with that of bevacizumab. The best conclusion that one can draw from this study is that ziv-aflibercept appears to be neither dramatically better nor worse than bevacizumab; this underlines the need for more studies with different drug combinations and direct comparisons with bevacizumab-based therapies.

Phase III research. Because of the acceptable clinical activity of ziv-aflibercept when used in combination therapies,[28-31,35] a Phase III prospective, multinational, double-blind, parallel-arm trial (the VELOUR trial) was conducted in patients with mCRC who had experienced disease progression while receiving oxaliplatin-based chemotherapy (e.g., FOLFOX).[37]

The primary study endpoint was OS, as determined by the planned subgroup analysis. Secondary endpoints were PFS and response rate. A total of 1226 patients with mCRC and an ECOG score of 2 or less were randomly assigned in a 1:1 fashion to receive ziv-aflibercept (n = 612) or a placebo (n = 614) followed by FOLFIRI chemotherapy every two weeks. Patients were stratified based on prior bevacizumab treatment and performance status (an ECOG score of 0 or 1 versus a score of 2). The study had a power of 90% to detect a 20% improvement in OS in the ziv-aflibercept group. All patients had received prior oxaliplatin therapy.

Ziv-aflibercept was given as a 4-mg/kg i.v. infusion prior to FOLFIRI chemotherapy. The FOLFIRI regimen was administered according to the National Comprehensive Cancer Network (NCCN) colon cancer guideline (irinotecan hydrochloride 180 mg/m^2 i.v. on day 1 of each two-week cycle, to be administered concurrently with leucovorin 400 mg/m^2 i.v., followed by fluorouracil 400 mg/m^2 by i.v. bolus and then fluorouracil 2400 mg/m^2 by continuous i.v. infusion over 46 hours[38]). The use of supportive care medications, including atropine (for prophylaxis of acute diarrhea due to irinotecan) and colony-stimulating factor (for prophylaxis of neutropenia) was permitted. Disease response was evaluated radiologically every six weeks. Treatment continued until disease progression or intolerable toxicity. The study groups were well balanced in terms of performance status and prior bevacizumab therapy. Approximately 30% of patients (186 of 612) in the ziv-aflibercept group had received bevacizumab, compared with 31% of patients (187 of 614) in the placebo group. The overall median age was 61 years; the majority of patients (87%) were white, 7% were Asians, and 3.5% were black.

At a median follow-up of 22.3 months, the two-year survival rates were 28.0% and 18.7% in the ziv-aflibercept and control groups, respectively. Relative to placebo users, patients treated with ziv-aflibercept had longer OS (13.5 months versus 12.1 months; hazard ratio, 0.817; 95% confidence interval, 0.71–0.94 months; p = 0.0032), a higher response rate (19.8% versus 11.1%, p < 0.001), and longer PFS (6.9 months versus 4.7 months, p < 0.0001). Patients receiving antiangiogenic therapy were given a median of seven cycles of ziv-aflibercept (the numbers of cycles of FOLFIRI and ziv-aflibercept varied among these patients); those in the control group received eight cycles

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of placebo administration. Irinotecan and fluorouracil were administered for a median of 21 weeks in the ziv-aflibercept group and 18.1 weeks in the control group.

Cycle delays and dose modifications were more common among patients receiving ziv-aflibercept. Dose intensity (defined as the ratio of the delivered chemotherapy dose to the standard full dose, expressed as a percentage) was 83% in the ziv-aflibercept group versus 92% in the control group. No significant relationship was observed between treatment outcomes and prior bevacizumab exposure. In a subgroup analysis, a greater OS benefit related to ziv-aflibercept therapy was observed only in patients with liver metastasis as opposed to metastasis to other organs. The benefits in OS and PFS seen in the bevacizumab-pretreated group were consistent with those observed in the overall population.

Adverse events among VELOUR trial participants included diarrhea, asthenia, stomatitis, ulceration, nausea, and infections. These adverse events were primarily grade 1 or 2 and were more common in patients treated with ziv-aflibercept.

Overall, adverse events of all grades were similar in both study groups. Grade 3 or 4 adverse events related to antiangiogenesis therapy that occurred more frequently in the ziv-aflibercept group relative to the control group included hypertension (19% versus 1.5%), hemorrhage (2.9% versus 1.7%), arterial thromboembolic events (1.8% versus 0.5%), venous thromboembolic events (8% versus 6%), and proteinuria (8% versus 1%). Surprisingly, a significant increase in chemotherapy-induced grade 3 or 4 toxicities—particularly diarrhea (19% versus 8%), asthenia (17% versus 11%), stomatitis (13% versus 5%), and infections (12% versus 7%)—was also observed with antiangiogenesis therapy (Table 2). This apparent increase in ziv-aflibercept-associated toxicities was not in line with Phase III study results on bevacizumab use in patients with mCRC.[38] However, prior bevacizumab treatment did not seem to impact the safety profile of ziv-aflibercept. The most common adverse events that led to treatment discontinuation included asthenia, infections, diarrhea, and hypertension.

Based on the results that demonstrated statistically significant improvement in OS, PFS, and response rate in patients with mCRC who received ziv-aflibercept as a second-line therapy with or without prior bevacizumab therapy, FDA approved ziv-aflibercept for use in this patient population in 2012.[22]

Safety

Overall, ziv-aflibercept appears to be tolerated, even in heavily pretreated mCRC populations. In the VELOUR trial, the most common adverse effects observed in patients receiving ziv-aflibercept in combination with the FOLFIRI regimen were leucopenia, neutropenia, thrombocytopenia, diarrhea, proteinuria, and hypertension.[22,37] These adverse effects were mostly grade 1 or 2 and reversible. Of note, some of these common adverse effects (hypertension and proteinuria) were among the most common DLTs identified in Phase I and II trials[26-29] and were consistent with the inhibition of the angiogenesis pathway. Other adverse effects occurring more commonly in ziv-aflibercept versus control groups (e.g., increases in liver function enzymes, stomatitis, fatigue, decreased appetite) may not be related to ziv-aflibercept's antiangiogenic effect.

Class-related adverse effects. Hypertension, proteinuria, and thromboembolism are recognized as the

Table 2.
Most Common Adverse Effects of Ziv-aflibercept in Phase III Trial[37,a]

Adverse Effect	% Patients Affected			
	FOLFIRI + Ziv-aflibercept (n = 611)		FOLFIRI + Placebo (n = 605)	
	All Grades	Grade 3 or 4	All Grades	Grade 3 or 4
Leukopenia	78	16	72	12
Neutropenia	67	37	57	30
Thrombocytopenia	48	3	35	2
Hypertension	41	19	11	2
Diarrhea	69	19	57	8
Stomatitis	50	13	33	5
Proteinuria	62	8	41	1
Fatigue	48	13	39	8
AST elevation	62	3	54	2
ALT elevation	50	3	39	2

[a]FOLFIRI = leucovorin, fluorouracil, and irinotecan; AST = aspartate transaminase; ALT = alanine transaminase.

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hallmark class-related adverse effects associated with antiangiogenic therapy.[39-42] Hypertension associated with antiangiogenic therapy is often of low grade (1 or 2). Even grade 3 or 4 hypertension may still be reversible, and patients are managed with angiotensin-converting enzyme inhibitors, calcium channel blockers, or diuretics.[43]

Proteinuria (all grades) was reported in 49% of patients in the VELOUR trial.[37] The frequency of grade 3 or 4 proteinuria was 7.8%; dose modification may be necessary for patients who develop this complication. The drug manufacturer recommends monitoring for proteinuria by urine dipstick (or urinalysis) and determination of the urinary protein-to-creatinine ratio (UPCR) prior to each dose of ziv-aflibercept.[22] For patients with a UPCR greater than 1, analysis of a 24-hour urine collection is recommended.

In addition, inhibition of the VEGF signaling pathway leads to dysfunctional endothelial cells in the vascular endothelium, causing activation of tissue factor and an increased risk of thrombosis. Experience with bevacizumab demonstrated that bevacizumab–VEGF complexes induced platelet aggregation and led to the thromboembolic events observed in clinical trials.[23,44] Currently, it remains unclear whether the biologically inert ziv-aflibercept–VEGF complex is associated with a lower frequency of thromboembolic events. In the VELOUR trial, grade 3 or 4 thromboembolic complications occurred in less than 1% of patients.[37] It is, however, important to note that cancer patients in general are at an increased risk for thrombosis due to their procoagulant disease state and that antiangiogenic therapy marginally increases this risk.[44]

Black-box warning. Ziv-aflibercept carries an FDA-mandated black-box warning on treatment-related hemorrhage, gastrointestinal perforation, and compromised wound healing.[22] In clinical trials, the rate of bleeding and hemorrhage together was 37.8% in the ziv-aflibercept groups versus 19% in the control groups[35,37]; the occurrence of grade 3 or 4 hemorrhage, including severe intracranial hemorrhage, was also slightly higher with ziv-aflibercept use (3% versus 1.7%).[22] Patients receiving ziv-aflibercept should be monitored for signs and symptoms of bleeding. Therapy should be discontinued in patients who develop severe hemorrhage. Although the rates of gastrointestinal perforation were similar in both study groups in the VELOUR trial, patients receiving antiangiogenic therapy should be monitored and treatment should be discontinued if gastrointestinal perforation develops.

Pregnancy consideration. Ziv-aflibercept is classified as a pregnancy category D agent. It should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.[22] Both female and male patients of reproductive potential must use effective contraception during treatment and for a minimum of three months after the last dose of ziv-aflibercept.

Immunogenicity and hypersensitivity. During clinical research, the frequency of development of antibodies to ziv-aflibercept was 3.1% in patients receiving ziv-aflibercept versus 1.7% in patients receiving a placebo[22]; among patients who tested positive for anti-ziv-aflibercept antibody, neutralizing antibodies were detected in 17 of 48 patients administered ziv-aflibercept and in 2 of 40 placebo users. The impact of neutralizing antibodies on treatment efficacy and safety remains unclear. Since the antibody assay may be influenced by many factors, direct comparison of the frequency of anti-ziv-aflibercept antibody formation with the corresponding rate for other protein-based medications may be misleading. Currently, it is not known if such antibody formation is persistent enough to cause infusion-related reactions or reduced ziv-aflibercept exposure. No data are available on the usefulness of routine screening for positive antibody.

In regard to the frequency of severe (grade 3 or 4) hypersensitivity reactions, no difference was found in ziv-aflibercept versus control patients in the VELOUR trial (0.3% versus 0.5%).[37]

Other safety considerations. Ziv-aflibercept, known simply as aflibercept in European and other countries, is specifically named with the prefix "ziv" (derived from the brand name Zaltrap and the abbreviation i.v.) in the United States in accordance with the FDA requirement that drug manufacturers reduce the potential for confusion related to sound-alike and look-alike medication names. The World Health Organization's international nonproprietary name for Zaltrap is aflibercept.

FDA required that the U.S. generic name ziv-aflibercept be used to clearly differentiate the formulation approved for use in managing mCRC from its ophthalmic counterpart, aflibercept, which is marketed under the proprietary name Eylea (Regeneron Pharmaceuticals) in a different strength.[22] The scientific literature still uses aflibercept as the generic name for ziv-aflibercept. It is recommended that ziv-aflibercept be stored, segregated, and labeled with a specific warning to help distinguish the product from the ophthalmic product.

Dosage, preparation, and administration

The recommended dosage of ziv-aflibercept is 4 mg/kg i.v. administered every two weeks prior to FOLFIRI combination chemotherapy. Treatment is continued until disease progression or intolerable toxicity. Ziv-aflibercept is supplied as a ready-to-use single-dose vial

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of either 200 or 100 mg (solution concentration, 25 mg/mL). After the withdrawal of the calculated dose from the vial, the drug is further diluted in a vehicle of 0.9% sodium chloride injection or 5% dextrose injection to yield a final ziv-aflibercept concentration of 0.6–8 mg/mL. Diluted solution must be refrigerated and used within four hours.[22]

Ziv-aflibercept should be administered as an intermittent infusion over one hour through a 0.2-μm polyethersulfone filter.[22] An administration set made of one of the following materials may be used: polyvinyl chloride (PVC)-containing diethylhexyl phthalate (DEHP), DEHP-free PVC containing trioctyl trimellitate, polypropylene, polyethylene-lined PVC, and polyurethane. Ziv-aflibercept is administered alone prior to any component of FOLFIRI combination chemotherapy.

Ziv-aflibercept has not been shown to have clinically significant interactions with irinotecan, SN-38, and fluorouracil.[26,37] The manufacturer has not conducted dedicated drug–drug interaction studies.[22]

The dosage of ziv-aflibercept does not need to be adjusted for renal impairment.[22] The VELOUR trial data indicated that no dosage adjustment is needed for mild hepatic impairment; however, no information is available on the potential need for dosage adjustments in severe hepatic impairment (defined in the VELOUR trial as a total bilirubin level greater than three times the upper limit of normal).[37]

Ziv-aflibercept should be discontinued for any of the following toxicities: arterial thromboembolic events, fistula formation, gastrointestinal perforation, severe hemorrhage, hypertensive crisis, hypertensive encephalopathy, nephrotic syndrome, thrombotic microangiopathy, and reversible posterior leukoencephalopathy syndrome.[22,44]

Dosage modifications should be made for patients with severe or uncontrolled hypertension; ziv-aflibercept should be withheld until blood pressure is controlled, with the dose subsequently reduced to 2 mg/kg.[22] For patient with substantial proteinuria (i.e., ≥2 g in 24 hours), ziv-aflibercept should be withheld and only administered when the protein level declines below that level. If proteinuria recurs, ziv-aflibercept must be discontinued until the urine protein level falls below 2 g in 24 hours, with therapy resumed at a reduced dose of 2 mg/kg.

Patients must be monitored for hematologic toxicity at baseline and prior to each cycle of ziv-aflibercept.[22] Treatment with ziv-aflibercept and FOLFIRI chemotherapy must be delayed until the neutrophil count is $\geq 1.5 \times 10^9$ cells/L. Furthermore, ziv-aflibercept has been shown to delay and compromise wound healing. Treatment should be stopped at least four weeks prior to elective surgery and should not be resumed until at least four weeks after surgery or until the surgical wound is fully healed.

No dosage adjustment is necessary for the elderly. Based on a population pharmacokinetic analysis,[22] race and sex did not have a clinically significant effect on exposure to free ziv-aflibercept. Currently, there is no recommendation for ziv-aflibercept dosing in obese patients. In one of the Phase I studies summarized above, patients weighing more than 100 kg had a 29% increase in systemic exposure to ziv-aflibercept compared with patients weighing 50–100 kg.[35] Furthermore, no recommendation for a maximum total dose of ziv-aflibercept has been made. In the VELOUR trial, a maximum patient weight was not specified.[22,37]

The use of FOLFIRI combination chemotherapy requires the calculation of body surface area (BSA).[22] In the VELOUR trial, if a patient's BSA was greater than 2 m^2, the amounts of irinotecan and fluorouracil used were adjusted downward to the doses corresponding to a BSA of 2 m^2 for safety purposes.[22,37]

Cost considerations

The average wholesale acquisition cost (WAC) of ziv-aflibercept is $1,600 per 100-mg vial or $3,200 per 200-mg vial.[45] Recently, the drug manufacturer discounted the cost by 50% for hospitals and clinics through its wholesalers and through group purchasing organizations. With a dose of 4 mg/kg and an average patient weight of 72 kg, an estimated dose for each treatment would be about 288 mg. Thus, each dose would cost approximately $4,800. Based on the clinical trial data, the median treatment duration of ziv-aflibercept was 6.9 months (PFS).[37] The cost of ziv-aflibercept alone for this treatment course is about $66,240. Since the drug was approved as an add-on to the standard second-line chemotherapy combination for mCRC (FOLFIRI), the total cost of the ziv-aflibercept–FOLFIRI regimen is approximately $80,000 for a patient with an average BSA (1.73 m^2). It is apparent that the use of ziv-aflibercept will result in a significant increase in overall mCRC treatment expenditure that may raise concerns among payers about the growing cost of oncology treatment.

On the other hand, the WAC of bevacizumab is $596 for a 100-mg vial or $2387 for a 400-mg vial.[45] Given the recommended dose of 5 mg/kg with standard chemotherapy, each dose of bevacizumab costs approximately $2387—only half the cost of ziv-aflibercept. The significant difference in cost between bevacizumab and ziv-aflibercept has brought renewed attention to cost issues in cancer care.[46] Whether an increased cost is justified by additional clinical efficacy is a robust area of comparative effectiveness research that is likely to continue.

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Place in therapy and future directions

The Phase III VELOUR trial demonstrated a statistically significant OS benefit, with improved PFS, in favor of ziv-aflibercept in combination with FOLFIRI chemotherapy relative to FOLFIRI chemotherapy alone in patients with mCRC that has progressed with an oxaliplatin-based regimen.[37] Common adverse effects are related to the mechanism-based effect of antiangiogenic therapy. Currently, there are no data to suggest that FOLFIRI plus ziv-aflibercept therapy has significant antitumor activity in patients whose disease has progressed during treatment with FOLFIRI plus bevacizumab. Ziv-aflibercept has only been shown to have antitumor activity when given in combination with FOLFIRI in FOLFIRI-naive patients. Therefore, NCCN recommends ziv-aflibercept as a second-line option to be used in combination with FOLFIRI or irinotecan only in patients with disease progression while receiving therapy not containing irinotecan.[38]

Considerable strides have been made regarding the use of bevacizumab beyond disease progression in the metastatic setting. In the setting of second-line chemotherapy that does not consist of FOLFIRI, current opinion seems to favor bevacizumab.[47-50] Ziv-aflibercept has yet to show safety and efficacy with chemotherapy regimens other than FOLFIRI in the second-line setting. Recently, a large Phase II trial (AFFIRM) of FOLFOX with or without ziv-aflibercept therapy for first-line treatment demonstrated no improvement in efficacy outcomes with ziv-aflibercept use.[51] In contrast, the activity of bevacizumab in the second-line treatment of patients with disease progression during first-line or even maintenance bevacizumab therapy is supported by some studies.[47-50] Similar efficacy, however, cannot be assumed for ziv-aflibercept based on current data.[22,37]

Ziv-aflibercept has the broad pharmacologic advantage of targeting endogenous ligands that are pro-angiogenic: VEGF-A, VEGF-B, and PlGF. How much of this advantage is translated to real clinical benefits is yet to be determined. On the other hand, the survival benefits conferred by bevacizumab have been clearly demonstrated in first-line, second-line, and maintenance treatment settings.[47-50] Bevacizumab also seems to have a more favorable safety profile than ziv-aflibercept (i.e., a lower potential to induce proteinuria and minimal constitutional toxicities).[23]

Ziv-aflibercept is appropriate for use as an add-on therapy in combination with irinotecan-based regimens in patients with mCRC who have not received irinotecan previously. At the time of writing, it cannot be recommended as first-line therapy, but an ongoing trial (NCT016522196) that is investigating the role of ziv-aflibercept in combination with FOLFOX chemotherapy in patients with previously untreated mCRC may shed light on the place of therapy for ziv-aflibercept.[52]

Like bevacizumab, ziv-aflibercept may be complementary to other cytotoxic chemotherapy agents. It has been studied in several tumor types and may be useful in various combinations.[26-31,40] Its distinctive mechanism of action may allow its use against other advanced solid tumors.[53] A Phase III trial of ziv-aflibercept plus docetaxel therapy for locally advanced or metastatic non-small-cell lung cancer demonstrated efficacy in terms of PFS and response rate but no major improvement in OS.[54] In another Phase III trial, combination therapy with ziv-aflibercept and gemcitabine was shown to produce no significant improvement in metastatic pancreatic cancer.[55] In metastatic prostate cancer refractory to hormonal therapy, recently published data from a Phase III study (the VENICE trial) showed that ziv-aflibercept plus docetaxel and prednisone failed to improve survival outcomes.[56] Until more study results are available, due to the aforementioned cost and reimbursement issues, ziv-aflibercept is indicated for selected patients with mCRC who have good performance status and whose disease is refractory to first-line oxaliplatin-based chemotherapy.

Conclusion

Current clinical data are insufficient to directly compare ziv-aflibercept and bevacizumab when used with standard combination chemotherapy as first- or second-line regimens for mCRC. The role of ziv-aflibercept is currently limited to the second-line setting in combination with irinotecan-based regimens in mCRC patients who have not received irinotecan previously. The role of ziv-aflibercept for other tumor types is yet to be determined.

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Increased Renal Expression of Vascular Endothelial Growth Factor (VEGF) and Its Receptor VEGFR-2 in Experimental Diabetes

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It has been suggested that the cytokine vascular endothelial growth factor (VEGF) has an important role in the pathogenesis of diabetic retinopathy, but its role in nephropathy has not been clearly demonstrated. Assessment of VEGF, ^{125}I-VEGF binding, and vascular endothelial growth factor receptor–2 (VEGFR-2) in the kidney was performed after 3 and 32 weeks of streptozotocin-induced diabetes. Gene expression of both VEGF and VEGFR-2 was assessed by Northern blot analysis and the localization of the ligand and receptor was examined by in situ hybridization. VEGF and VEGFR-2 protein were also evaluated by immunohistochemistry. Binding of the radioligand ^{125}I-VEGF was evaluated by in vitro and in vivo autoradiography. Diabetes was associated with increased renal VEGF gene expression. VEGF mRNA and protein were localized to the visceral epithelial cells of the glomerulus and to distal tubules and collecting ducts in both diabetic and nondiabetic rats. Renal VEGFR-2 mRNA was increased after 3 weeks of diabetes but not in long-term diabetes. In situ hybridization and immunohistochemical studies revealed that glomerular endothelial cells were the major site of VEGFR-2 expression. In addition, VEGFR-2 gene expression was detected in cortical and renomedullary interstitial cells and on endothelial cells of peritubular capillaries. There was an increase in ^{125}I-VEGF binding sites after 3 but not 32 weeks of diabetes. The major VEGF binding sites were in the glomeruli. ^{125}I-VEGF binding was also observed in medullary rays and in the renal papillae. These studies indicate an early and persistent increase in renal VEGF gene expression in association with experimental diabetes. In addition, an early and transient increase in renal VEGF receptors was also observed in diabetic rats. These findings are consistent with a role for VEGF in mediating some of the changes observed in the diabetic kidney. *Diabetes* 48:2229–2239, 1999

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is a term used to describe a family of potent multifunctional cytokines (1). The VEGF-A gene produces five closely related species by alternative splicing. VEGF-A_{165} is the most abundantly expressed species and is a 165–amino acid glycoprotein with 20% homology to the A and B chains of platelet-derived growth factor (1). At least two high-affinity receptor-binding sites have been reported for VEGF. These receptors have been cloned and named flt-1 (vascular endothelial growth factor receptor [VEGFR]-1) and flk-1/KDR/NYK (VEGFR-2) and were initially viewed to be endothelial-specific tyrosine kinases (2,3). More recently, it has been determined that these receptors are present on other cell types (4). Recent studies suggest that it is the biological interaction of VEGF with VEGFR-2 that is involved in inducing the spectrum of biological responses of VEGF (1,5). VEGF also has a selective action of promoting vascular permeability (6), being 50,000 times more potent than histamine on a molar basis (7). Radioligand binding studies with iodinated recombinant VEGF have confirmed a wide distribution of VEGF binding sites in the rat, including the kidney (8). More recently, VEGF binding has also been detected in the adult and fetal human kidney (9).

Recent studies have emphasized the potential role of VEGF in diabetic retinopathy (10,11), with several groups reporting an increase in VEGF and its receptors in the diabetic retina (12,13). It has been shown that there is a close relationship between proliferative diabetic retinopathy and nephropathy (14). Furthermore, several of the putative pathways implicated in the pathogenesis of diabetic retinopathy are also viewed to be involved in the development of diabetic nephropathy (15,16). However, the status of VEGF and its receptors in the kidney in experimental diabetes has not been previously characterized.

RESEARCH DESIGN AND METHODS

Experimental plan. Diabetes was induced by streptozotocin (Sigma, St. Louis, MO) injection (diabetic, 60 mg/kg i.v.) or sham injection with citrate buffer injection (control) after an overnight fast in Sprague-Dawley rats weighing 200–250 g. Before the rats were killed, blood was collected for determination of plasma glucose by the glucose oxidase technique (17) and systolic blood pressure was measured by tail cuff plethysmography (18). The animals were anesthetized with

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AGE, advanced glycated end product; IC_{50}, concentration of unlabeled ligand required to displace 50% of the radioligand; NSB, nonspecific binding; PBS, phosphate-buffered saline; PKC, protein kinase C; ROD, relative optical density; SSC, sodium chloride–sodium citrate; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor–2.

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pentobarbital sodium (Nembutal 50 mg/kg body weight i.v.; Bomac Laboratories, Asquith, Australia) and killed after 3 or 32 weeks of diabetes. Both kidneys were then removed. One kidney was snap frozen in liquid nitrogen–cooled isopentane for use in extracting RNA for Northern hybridization and for in vitro autoradiographic binding studies. The other kidney was bissected and immediately fixed in freshly prepared 4% paraformaldehyde in preparation for in situ hybridization. A separate group of control and diabetic rats were prepared in a manner similar to that described above, except that both kidneys were removed after infusion of radioactive VEGF tracer for the in vivo autoradiographic binding studies.

Northern hybridization of VEGF and its receptors

Preparation of RNA and synthesis of probes. Total RNA was isolated from the kidney at the time when the animals were killed using the acid guanidium thiocynate-phenol-chloroform extraction method (19). RNA purity and concentration were determined spectrophotometrically. Twenty-microgram samples were denatured and electrophoresed through 0.8% agarose formaldehyde gels. RNA integrity was verified by examination of the 28S and 18S ribosomal bands of ethidium bromide–stained material under ultraviolet light. RNA was then transferred by capillary action onto nylon filters (Hybond-N; Amersham, Bucks, U.K.) and fixed under ultraviolet light. Filters were then probed with the cDNA coding for either VEGF or its receptors, VEGFR-1 and VEGFR-2 (13). The probes were then labeled with [^{32}P]dCTP by randomly primed DNA synthesis (Boehringer Mannheim, Mannheim, Germany). Hybridization was performed at 42°C for 16 h in 50% formamide, 45 mmol/l Na_2HPO_4, 5× Denhardt's solution, 0.5% SDS, and sonicated salmon sperm DNA. After hybridization, filters were washed in solutions of decreasing ionic strength and increasing temperature, followed by exposure of filters to X-ray film for 5–14 days.

In situ hybridization of VEGF and VEGFR-2

Synthesis of riboprobes. Sense and antisense RNA probes for VEGF and VEGFR-2 were generated by in vitro transcription (Promega, Madison, WI), as previously described (13). In brief, linearized template (500 ng) was added to a reaction mixture of transcription buffer (final volume 20 µl) containing 6 mmol/l dithiothreitol, 333 µmol/l each of ATP, CTP, and GTP, 12 µmol/l UTP, 100 µCi [^{33}P]UTP (2,000 Ci/mmol, New-Dupont, Boston, MA), 20 U RNAsin (Boehringer-Mannheim), and 20 U RNA polymerase (Boehringer-Mannheim). The reaction mixture was incubated at 37°C for 90 min, after which the DNA template was digested with 1 U RNAse-free DNAse for 15 min. The riboprobe was precipitated by ammonium acetate and ethanol using yeast tRNA as a carrier and then reconstituted in 100 µl of water. Purified riboprobe length was adjusted to ~150 bases by alkaline hydrolysis.

In situ hybridization. Four-micrometer-thick sections cut from paraformaldehyde-fixed paraffin-embedded kidney tissue were placed onto slides precoated with 3-aminopropyltriethoxysilane and left overnight at 37°C. Tissue sections were dewaxed in histolene, rehydrated in graded ethanols to deionized milliQ water, equilibrated in P buffer (50 mmol/l Tris-HCl, pH 7.5, 5 mmol/l EDTA), and incubated in 125 µg/ml Pronase E (Sigma) in P buffer for 10 min at 37°C. Sections were then washed in 0.1 mol/l sodium phosphate buffer (pH 7.2), briefly refixed in 4% paraformaldehyde for 10 min, rinsed in milliQ water, dehydrated in 70% ethanol, and air dried.

Hybridization buffer containing 2×10^4 cpm/µl riboprobe in 300 mmol/l NaCl, 10 mmol/l Tris-HCl (pH 7.5), 10 mmol/l Na_2HPO_4, 5 mmol/l EDTA (pH 8.0), 1× Denhardt's solution, 50% formamide, 17 mg/ml yeast RNA, and 10% wt/vol dextran sulfate was heated to 85°C for 5 min. A 25-µl aliquot of this solution was then applied to each tissue section under coverslips. Hybridization of tissue to the riboprobe was performed overnight at 60°C in 50% formamide-humidified chambers. Sense probes for VEGF and its receptor, neuroepithelial tyrosine kinase (NYK), were used on a further set of tissue sections as controls for nonspecific binding.

After hybridization, slides were washed in 2× sodium chloride–sodium citrate (SSC) containing 50% formamide prewarmed to 50°C to remove coverslips. Sections were then washed in the above-mentioned solution for 1 h at 55°C, rinsed three times in RNAse buffer (10 mmol/l Tris-HCl, pH 7.5, 1 mmol/l EDTA, pH 8.0, 0.5 mol/l NaCl), and then incubated with RNAse A (150 µg/ml) for 1 h at 37°C. Sections were later washed in 2× SSC for 45 min at 55°C, dehydrated in graded ethanol, air dried, and exposed to Kodak X-Omat autoradiographic film (Kodak, Rochester, NY) for 1–3 days. Slides were then dipped in Ilford K5 nuclear emulsion (Ilford, Mobberley, U.K.), stored in a light-free box with desiccant at room temperature for 2–3 weeks, and developed in Kodak D19, followed by fixation with Ilford Hypam. Sections were then stained with hematoxylin and eosin for examination under light microscopy.

Quantitation of Northern and in situ hybridization X-ray films. The relative intensity of autoradiograms of the Northern blots was determined by scanning densitometry (Ultrocan XL; LKB, Bromma, Sweden). All results were corrected for differences in RNA loading by rehybridization with an oligonucleotide probe for 18S rRNA. Densitometry of autoradiographic images from kidney sections, obtained by in situ hybridization, was performed as previously described (20), using a Micro Computer Imaging Device (MCID; Imaging Research, St. Catharines, Ontario, Canada), according to the methods of Baskin and Stahl (21). In brief, in situ autoradiographic images were placed on a uniformly illuminating fluorescent light box (Northern Light Precision Luminator Model C60; Image Research) and captured using a video camera (Sony Video Camera Module CCD; Tokyo) connected to an IBM AT computer with a 512–by-512 pixel array imaging board with 256 gray levels. In view of the focal nature of glomerular [^{125}I]VEGF binding, the mean of 20–25 quantitations of these areas was assessed for each kidney section (22). After calibration, construction of a curve of optical versus radioactivity density was possible and quantitation of digitalized autoradiographic images is performed using MCID software (22). Results were then expressed as relative optical density (ROD) (23). All measurements were performed by an independent observer (B.R.) in a masked manner.

Immunohistochemistry. Sections 4 µm thick were placed onto slides, rehydrated, and treated with 1% H_2O_2/methanol followed by incubation in Protein Blocking Agent (Lipshaw-Immunon, Pittsburgh, PA) for 20 min at room temperature, as previously described (13). Sections were next incubated with a polyclonal rabbit antibody directed against the NH_2-terminal 21 amino acids of human VEGF (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C, washed in phosphate-buffered saline (PBS), and incubated with biotinylated goat anti–rabbit igG (DAKO, Carpinteria, CA). After washing with PBS, sections were incubated with avidin-biotin complex (Vector, Burlingame, CA). Peroxidase conjugates were subsequently localized by diaminobenzidine tetrahydrochloride as a chromogen. Sections were counterstained with hematoxylin. Antibody specificity had been previously confirmed by preabsorption of antibody with recombinant VEGF and by incubation without the primary antiserum (13). A similar procedure was used for assessment of VEGFR-2 protein using a polyclonal rabbit antibody directed against the COOH-terminal 20 amino acids of the mouse flk-1 protein (3).

VEGF binding in the kidney

Iodination of VEGF-165. Recombinant human VEGF (R + D Systems, Minneapolis, MN) (0.5 µg) was iodinated with ^{125}I using the chloramine T method (24). A 5-µl (0.5 mCi) aliquot of ^{125}I was added to the recombinant VEGF. Next, 50 µl of Chloramine T at a concentration of 5 mg/10 ml was added and the mixture allowed to react for 60 s. To halt the reaction, 50 µl of sodium metabisulphite (5 mg/ml) was added. After iodination, the labeled protein was separated on a Sep-pak C18 column (Millipore, Milford, MA).

In vitro autoradiography. Frozen 20-µm sections were cut on a cryostat and placed onto gelatin-coated slides. Tissue sections were washed twice for 7.5 min in preincubation buffer containing 10 mmol/l HEPES, 100 mmol/l NaCl, 5 mmol/l KCl, 5 mmol/l $MgCl_2$, 1 mmol/l EGTA, 0.1% bovine serum albumin, and 0.5 mg/ml bacitracin. Tissue sections were then incubated for 3 h in incubation buffer, consisting of preincubation buffer and ^{125}I-VEGF tracer. The final amount of radioactivity in the incubation buffer was ~ 300 cpm/µl; a total of 2.4×10^6 cpm was used with a final concentration of ~2 pmol/l ^{125}I-VEGF. Tissue sections were washed first in preincubation buffer for 4 min, followed by two 4-min washes in postincubation buffer consisting of 10 mmol/l HEPES, 100 mmol/l NaCl, 5 mmol/l KCl, 5 mmol/l $MgCl_2$, 1 mmol/l EGTA, and 0.5 mg/ml bacitracin. Once sections were dry, they were placed against X-ray film (Kodak X-o-Mat; Kodak, Rochester, NY) for 2 weeks. Competitive binding studies were performed with varying concentrations (10^{-12} to 10^{-9} mol/l) of unlabeled VEGF. Nonspecific binding was determined by addition of 1 nmol/l of unlabeled VEGF. Radioactive standards were made by using known dilutions of ^{125}I-VEGF onto standard disks of rat kidney slices, which were included in each slide cassette to allow calculation of autoradiographic density into units of radioactivity, as previously described (22). Quantitation of binding was performed using a computerized imaging system (MCID; Imaging Research), and one kidney from five rats per group was examined. By means of this imaging system, the density of radioactive VEGF that binds to each kidney section can be calculated. In view of the focal nature of ^{125}I-detected rat VEGF glomerular binding, the mean of 20–25 quantitations of these areas was assessed for each kidney section (22). At least five sections per rat were analyzed. The data were then analyzed by SIGMAPLOT (Jandel Scientific, San Rafael, CA) to yield a value for the concentration of unlabeled ligand required to displace 50% of the radioligand ^{125}I-VEGF (IC_{50}) (22).

In vivo autoradiography. To further define cellular localization of ^{125}I-VEGF binding in the renal cortex, the tracer was infused in vivo in separate groups of rats (control, $n = 6$; diabetic, $n = 5$). This approach has been used when characterizing other binding sites, since it is associated with better preservation of renal morphology (25,26). Rats were anesthetized with pentobarbital sodium (Nembutal 50 mg/kg body weight) and the abdominal cavity was opened with a midline incision. For infusion of ^{125}I-VEGF, a 22-gauge catheter was inserted into the abdominal aorta below the renal arteries. For total binding studies, rats were infused with ~15 µCi ^{125}I-VEGF in 1 ml of 0.1 mol/l PBS at pH 7.4. After 10–15 min of circulation, the rats were perfused at systolic blood pressure (~140 mmHg) with PBS for 1 min followed by 2% glutaraldehyde for 2 min. After perfusion fixation, one kidney was removed and fixed in 10% neutral buffered formalin overnight before it was embedded in paraffin and sections pre-

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TABLE 1
Metabolic parameters

	Short-term (3 weeks)		Long-term (32 weeks)	
	Control	Diabetic	Control	Diabetic
n	10	6	10	8
Glucose (mmol/l)	7.9 ± 0.4	17.7 ± 1.2*	8.2 ± 0.4	19.8 ± 1.5*
Blood pressure (mmHg)	129 ± 4	122 ± 8	135 ± 3	137 ± 2
Body weight (g)				
Week 0	232 ± 5	228 ± 8	230 ± 4	220 ± 6
End of study	316 ± 12	220 ± 6*	495 ± 16	344 ± 9*

Data are means ± SE. *P < 0.01 vs. control.

pared for emulsion autoradiography. Nonspecific binding (NSB) was determined in separate animals by infusion of excess (10^{-9} mol/l) unlabeled VEGF proteins 3 min before infusion of ^{125}I-VEGF tracer. The procedure was then followed as described above. The other kidney was frozen in liquid nitrogen–cooled isopentane, and 20-μm sections were cut on a cryostat and then dried and placed against X-ray film. Between 8 and 13 sections per animal were quantitated using the MCID imaging system as described above.

Statistical analysis. All data were compared by analysis of variance using the statistical package Statview SE+Graphics on an Apple Macintosh computer (Apple Computer, Cupertino, CA) and are shown as means ± SE. Differences between groups were assessed using Fisher's least significant differences test (27).

RESULTS

At the end of both short-term (3 weeks) and long-term (32 weeks) studies, all streptozotocin-induced diabetic animals had hyperglycemia (plasma glucose >15 mmol/l) and decreased body weight compared with the control groups (Table 1). In the short-term diabetic group, no weight gain was observed over the 3-week period. No difference in blood pressure was observed between control and diabetic groups.

Northern analysis. Representative Northern hybridization blots for both VEGF and VEGFR-2 in the short-term and long-term studies are shown in Fig. 1. By Northern analysis, short-term diabetes was associated with a threefold increase in VEGF mRNA when compared with control rats (Fig. 2*A*). There was also a twofold increase in VEGFR-2 gene expression with short-term diabetes (Fig. 2*A*). Long-term diabetes was associated with a twofold increase in VEGF mRNA (Fig. 2*B*). VEGFR-2 mRNA levels were not different between con-

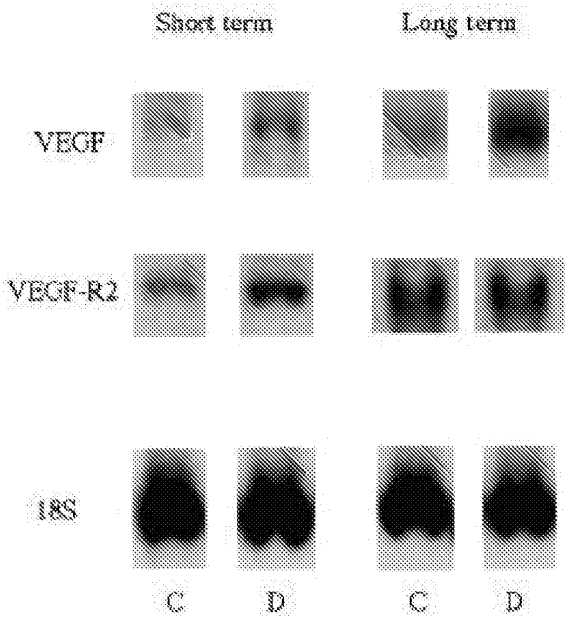


FIG. 1. Northern blot of VEGF, VEGFR-2, and 18S in control (C) and diabetic (D) rat kidneys. Increased gene expression of VEGF and VEGFR-2 is seen in kidneys of short-term diabetic rats. Increased gene expression of VEGF but not VEGFR-2 is seen in kidneys of long-term diabetic rats. 18S rRNA is similar in control and diabetic groups.

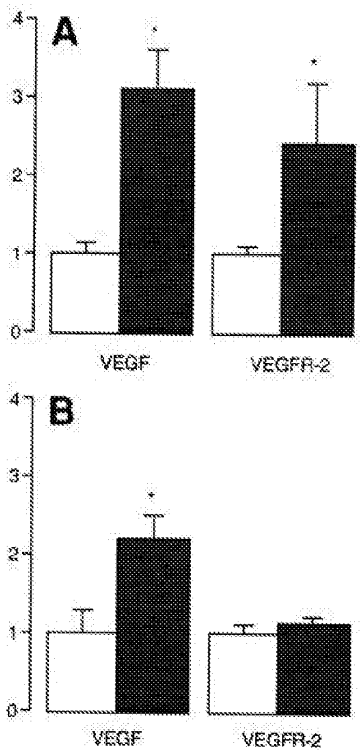


FIG. 2. Quantitation of kidney VEGF and VEGFR-2 mRNA in control (□) and diabetic (■) rats from short-term (*A*) and long-term (*B*) studies. Data are means ± SE of the ratio of optical density of VEGF and VEGFR-2 mRNA to that of 18S rRNA relative to control values (designated an arbitrary value of 1). *P < 0.01; diabetic versus control.

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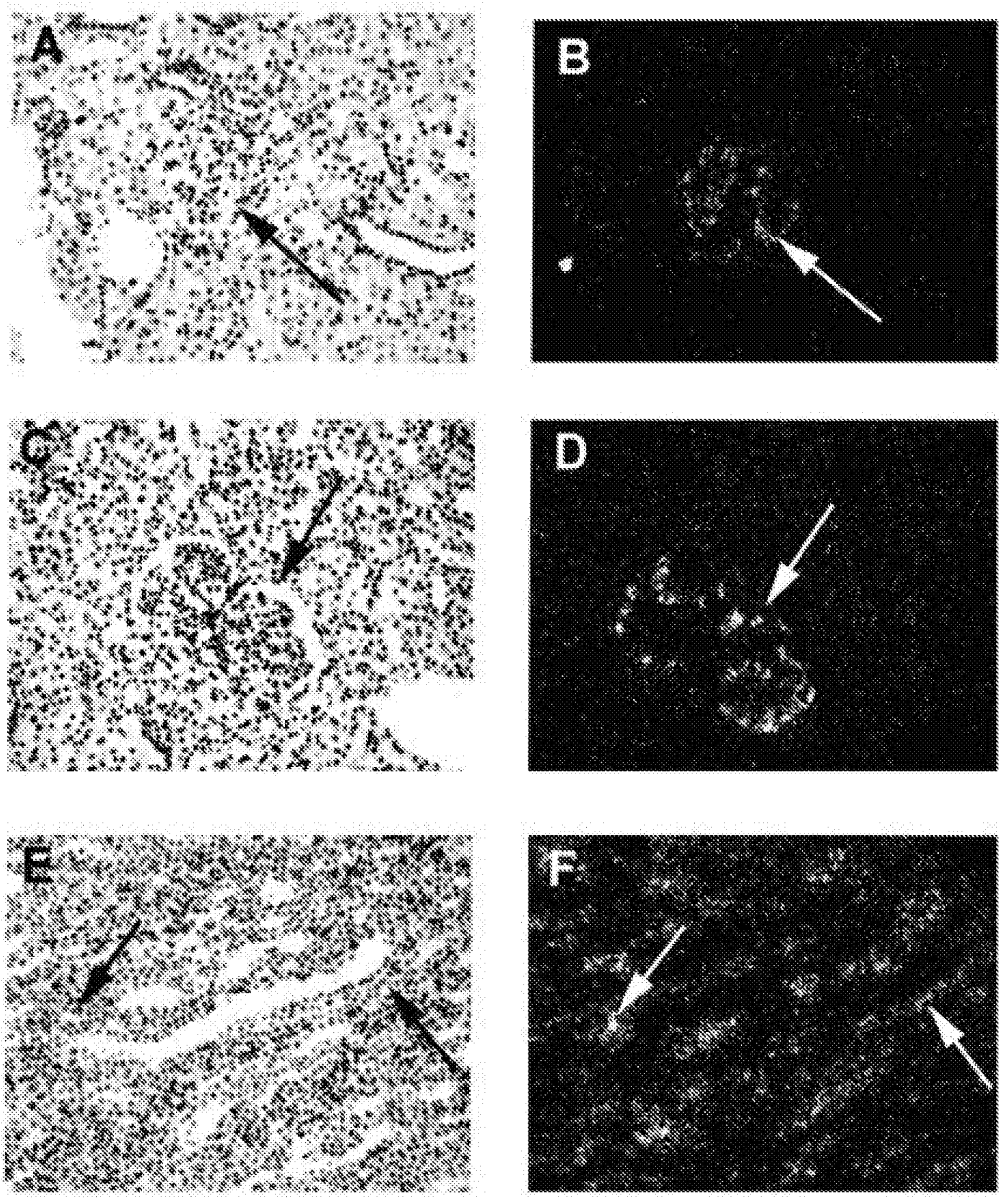


FIG. 3. In situ hybridization photomicrographs of VEGF mRNA. In the cortex, hybridization is present in the glomerular epithelial cells (arrow) in control (*A*: light field; *B*: dark field) and in greater abundance in diabetic (*C*: light field; *D*: dark field) rat kidneys. In both control and diabetic kidneys, collecting ducts expressed VEGF (control rats, *E*: light field; *F*: dark field). Original magnification 200×.

trol and diabetic rats (Fig. 2*B*). By contrast, there was no evidence of hybridization to a VEGFR-1 riboprobe in either control or diabetic rat kidney.

Localization of VEGF and VEGFR-2. Light microscopy following in situ hybridization of sections revealed abundant expression of VEGF mRNA in the visceral epithelial cells of glomeruli (Fig. 3*A–D*) and in distal tubules and collecting ducts (Fig. 3*E* and *F*). Gene expression for the VEGF receptor, VEGFR-2, was detected in glomerular endothelial cells (Fig. 4*A–D*). In addition, VEGFR-2 mRNA was noted in cortical interstitial fibroblasts and endothelial cells of peritubular capillaries (Fig. 4*E*) and renomedullary interstitial cells (Fig. 4*F*). No difference in the pattern of distribution between control and diabetic rats was noted for either VEGF (Fig. 3*A–D*) or VEGFR-2 mRNA (Fig. 4*A–D*). No hybridization was observed with sense probes (Fig. 5).

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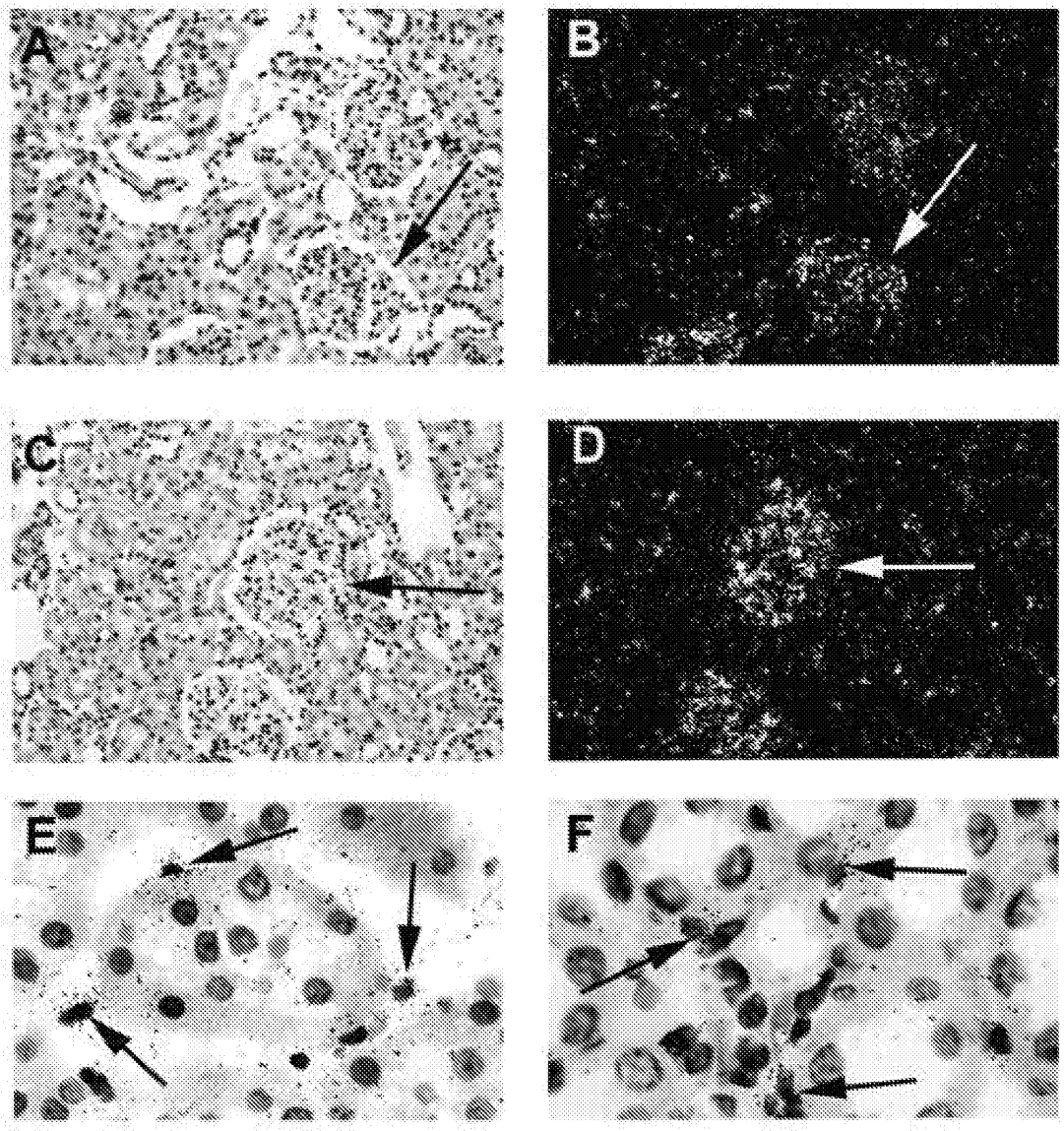


FIG. 4. In situ hybridization photomicrographs of VEGFR-2 mRNA. In the cortex, hybridization is present in the glomerular endothelial cells in control (*A*: light field; *B*: dark field) and in greater abundance in diabetic (*C*: light field; *D*: dark field) rat kidneys. Original magnification 200×. In both control and diabetic kidneys, VEGFR-2 was expressed in cortical fibroblasts, in endothelial cells of peritubular capillaries (*E*), and in renomedullary interstitial cells (*F*). Original magnification 1,000×.

Quantitation of in situ hybridization autoradiography. Kidney sections labeled with the VEGF probe showed that hybridization within glomeruli was more intense in the short-term diabetic group compared with the age-matched nondiabetic group (control, $n = 10$; 0.213 ± 0.006 vs. diabetic, $n = 6$; 0.314 ± 0.006, ROD units, mean ± SE, $P = 0.002$).

Quantitation of glomerular VEGF mRNA expression in long-term diabetic rats showed similar results to that observed in the short-term diabetic animals. Glomerular VEGF mRNA expression in the diabetic group was increased when compared with the nondiabetic group (control, $n = 5$; 0.247 ± 0.016 vs. diabetic, $n = 5$; 0.314 ± 0.020 ROD, $P = 0.03$).

Immunohistochemistry. VEGF protein was detected in glomerular epithelial cells, distal tubules, and collecting ducts (Fig. 6*A*). A similar distribution of VEGF protein was observed in diabetic rats, although the immunostaining appeared to be increased at these sites (Fig. 6*B*). There was only minimal VEGFR-2 protein detected in glomerular endothelial cells in control rats (Fig. 7*A*). Immunostaining of glomerular endothelial cells was increased in short-term diabetic rats (Fig. 7*B*).

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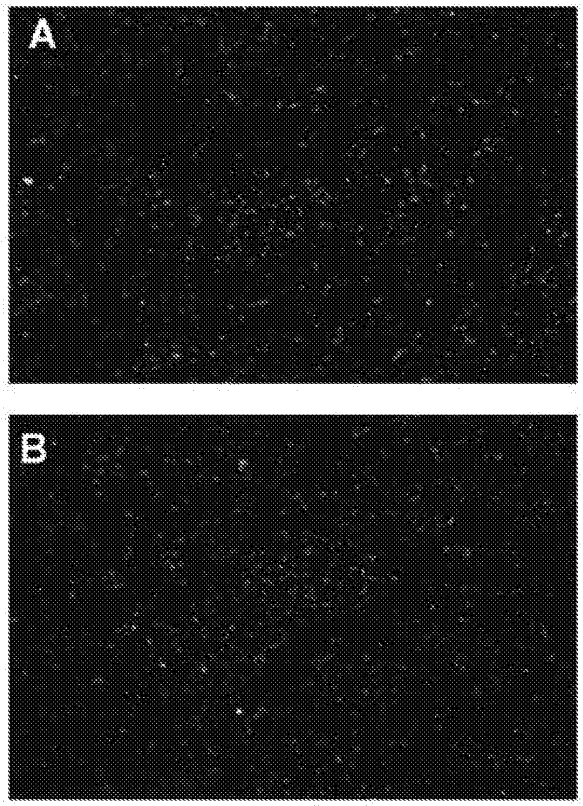


FIG. 5. In situ hybridization photomicrographs of sense VEGF (*A*) and VEGFR-2 (*B*) mRNA. No specific hybridization was detected in sections exposed to the VEGF and VEGFR-2 sense probes. Original magnification 300×.

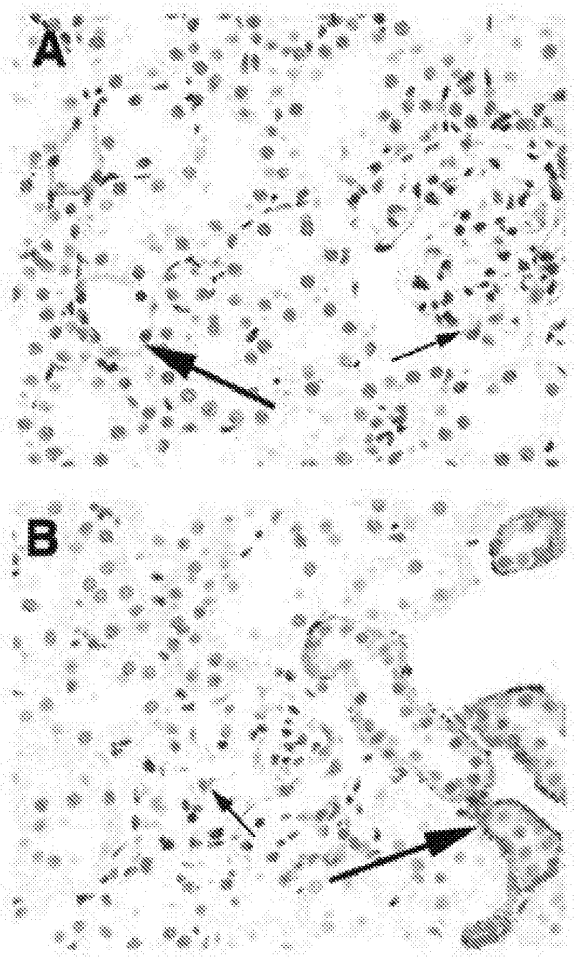


FIG. 6. Paraffin sections (4-μm thick) of control (*A*) and diabetic (*B*) rat kidney were immunostained for VEGF and counterstained with hematoxylin. Original magnification 320×. The arrows identify immunolabeling to distal tubules and glomerular epithelial cells. There appeared to be increased immunostaining in the diabetic kidney.

^{125}I-VEGF binding in the diabetic kidney

In vitro studies. Maximal receptor binding of iodinated VEGF-165 was seen after 5 h at a concentration of ~2 pmol/l (specific activity of 2,000 Ci/mmol). Binding of ^{125}I-VEGF was visualized by X-ray film in both control and diabetic rat kidney sections. In both control and diabetic rats, ^{125}I-VEGF binding was observed in the renal cortex, medulla, and papilla (Fig. 8).

Total binding of VEGF in the diabetic rats was increased by ~50% after 3 weeks of diabetes. Competition studies between radiolabeled VEGF and purified unlabeled VEGF were performed. ^{125}I-VEGF binding was inhibited by 10^{-9}mol/l unlabeled VEGF (i.e., NSB). NSB was <10% in all experiments (Fig. 8).

Cortex: short-term diabetes. Both control and diabetic kidneys showed similar ^{125}I-VEGF binding characteristics (Fig. 9*A*) ($-\log IC_{50}$; control, 11.14 ± 0.14 mol/l vs. diabetic, 11.21 ± 0.03 mol/l, n = 4/group). Renal cortical binding was increased in diabetes (control, 484 ± 11 dpm/mm^2; diabetic, 687 ± 18 dpm/mm^2, P = 0.001, n = 4/group).

Medulla. Medullary binding was also increased in the diabetic kidney (control, 368 ± 10 dpm/mm^2 vs. diabetic, 669 ± 18 dpm/mm^2, P < 0.001, n = 4/group). The binding curve, shown in Fig. 9*B*, was analyzed, and it showed that binding characteristics were modestly, yet statistically, different between the control and diabetic kidneys (control, $-\log IC_{50}$; 10.85 ± 0.16 mol/l; diabetic, 11.16 ± 0.08 mol/l, P < 0.001, n = 4/group).

In vivo binding studies. Localization of VEGF binding sites is shown in Fig. 10. There was increased glomerular binding in diabetic rats in comparison with control rats (control, 2,543 ± 53, n = 6 vs. 4,555 ± 134 dpm/mm^2, n = 5, P < 0.001).

DISCUSSION

The present study has documented increased expression of VEGF within the kidney after induction of diabetes. This increase persisted over the 8 months of diabetes. By means of in situ hybridization and immunohistochemical techniques, VEGF gene expression was localized to the epithelial cells of the glomerulus. This localization of VEGF is as pre-

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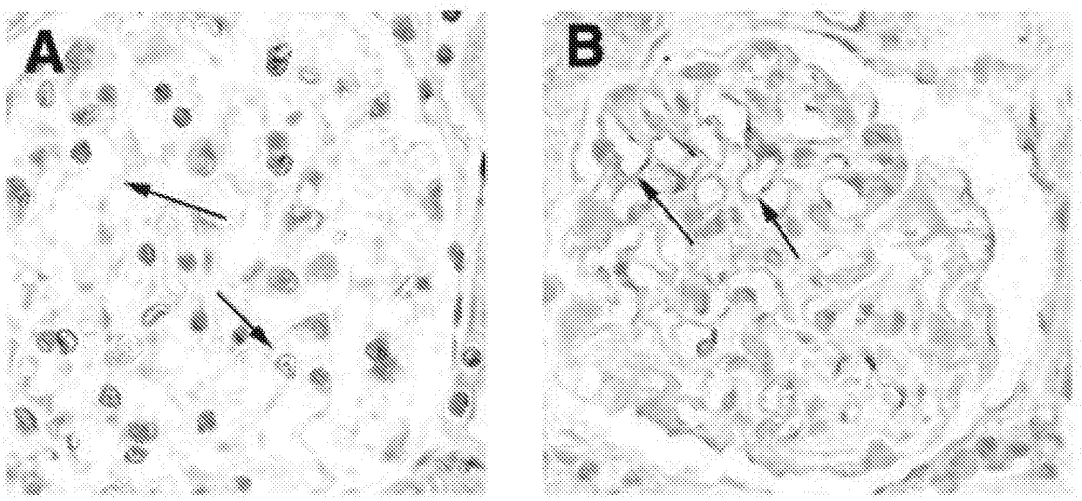


FIG. 7. Paraffin sections (4-μm thick) of control (*A*) and diabetic (*B*) rat kidney were immunostained for VEGFR-2 and counterstained with hematoxylin. Original magnification 380×. The arrows identify immunolabeling to glomerular endothelial cells. There is increased immunostaining in the diabetic kidney.

viously described by other groups and is similar to that reported in human kidney (9). In addition, VEGF was detected in collecting ducts, as has been previously reported (28). Although diabetes was associated with increased VEGF gene expression, as assessed by both Northern analysis and by quantitative evaluation of the in situ hybridization studies, there was no difference in the spatial distribution of glomerular VEGF between control and diabetic rats.

There was also increased gene expression of VEGFR-2 in the kidneys from animals with short-term diabetes. Furthermore, in long-term diabetic rats, VEGFR-2 expression was also noted in the interstitium, including both fibroblasts and peritubular capillaries. This receptor has been shown to mediate the full biological spectrum of actions of VEGF (1). This increase in receptor gene expression was readily observed in short-term diabetes but was not evident in long-term diabetic rats. Although this difference in renal receptor expression between short- and long-term diabetic rats may relate to effects of duration of diabetes, one cannot exclude that other factors, such as the severity of diabetes or low-dose insulin treatment, may also influence VEGF receptor expression.

In situ hybridization studies revealed that VEGFR-2 was expressed in glomeruli, as has been previously reported (28). The endothelial distribution of this VEGF receptor is consistent with the specific mitogenic effect of VEGF on endothelial cells (29). Immunohistochemical studies confirmed that there was translation of the VEGFR-2 mRNA to protein with evidence of receptor protein expression in glomerular endothelial cells, as has been previously reported (9). In the present study, VEGFR-2 was also detected in both control and diabetic rats in cortical fibroblasts and renomedullary interstitial cells. The significance of this finding is as yet unknown, but these cells lie in close proximity to tubular capillaries, and the action of VEGF at these sites may involve effects on vascular integrity. These interstitial cells have been reported to have receptors for many growth factors (30) and vasoactive hormones, such as angiotensin II and endothelin (31).

To complement the studies evaluating VEGFR-2 expression, both in vivo and in vitro autoradiographic techniques were used to assess ^{125}I-VEGF binding in control and diabetic rats. Specific VEGF binding sites were observed in the kidney, including the glomerulus, medullary rays, and papillae of the kidney, as has been previously described by Jakeman et al. (8). Further evaluation of ^{125}I-VEGF binding revealed an increase in specific binding in experimental diabetes consistent with translation of the VEGF receptor mRNA to receptor protein, thereby leading to increased binding of the ligand VEGF. The increase in VEGF binding suggested by in vitro autoradiography is consistent with the increased expression of VEGFR-2 detected in the diabetic kidney using both in situ hybridization and immunohistochemical techniques. These findings using three different methods support the notion that there is upregulation of VEGF receptors in the diabetic kidney.

In vivo autoradiography revealed a similar pattern to the in vitro studies with increased binding, primarily to glomeruli, in diabetes. Although the findings from the in vivo autoradiographic studies could be confounded by renal hemodynamic changes such as diabetes-related renal hyperperfusion-hyperfiltration, this is unlikely to explain the increased renal VEGF binding, since the in vitro autoradiographic studies, which are not confounded by systemic or renal hemodynamic factors, also showed increased binding of the radioligand ^{125}I-VEGF.

Although VEGFR-1 can also bind VEGF with high affinity, recent studies indicate that this protein does not initiate VEGF signal transduction pathways and is not primarily responsible for the biological actions of VEGF in the adult (5,32). This receptor has been previously reported to be present in the kidney (9). However, other groups have suggested that there is minimal or no expression of VEGFR-1 in the kidney (33–35). Indeed, in the present study, VEGFR-1 mRNA

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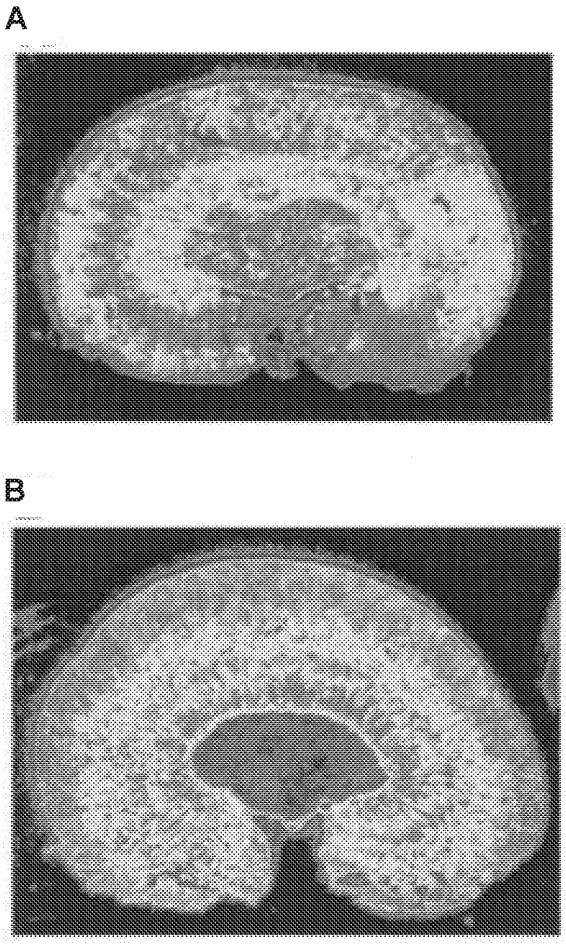


FIG. 8. Magnitude of ^{125}I-VEGF binding in the kidney from control (*A*) and diabetic (*B*) rats is indicated semiquantitatively in pseudocolorized computer images (blue, nil; green, low; yellow, moderate; red, high).

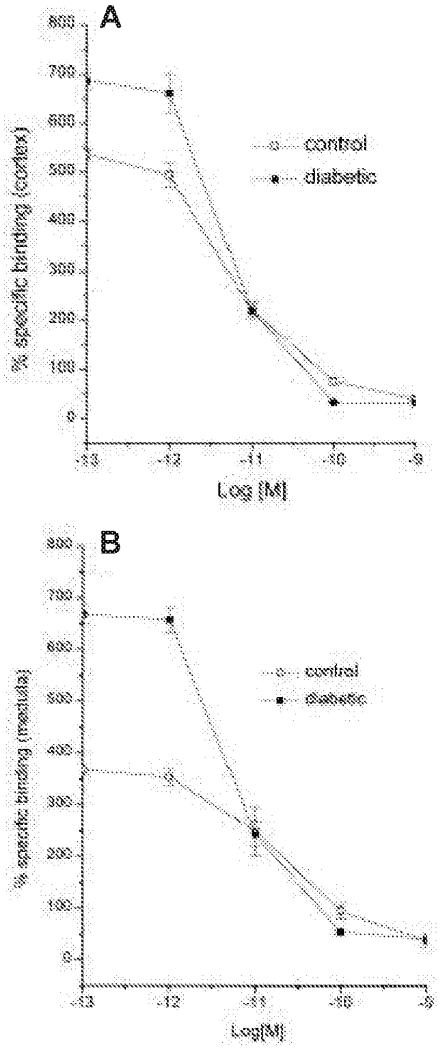


FIG. 9. Binding isotherms for VEGF generated by the incubation of tissue sections in [^{125}I]VEGF with increasing concentrations (10^{-12} to 10^{-9} mol/l) of nonradioactive rat VEGF in control (□, n = 5) and diabetic (■, n = 5) rats in renal cortex (*A*) and medulla (*B*). The y-axis represents percent specific binding.

could not be detected by Northern analysis in either control or diabetic rat kidney. By contrast, abundant VEGFR-1 mRNA expression was observed in rat lung and placenta (data not shown) and in retina, as has been previously reported by our group and others (12,13). It has been suggested that the dominant form of this receptor in the kidney is the soluble form that lacks an intracellular signaling domain (33). Nevertheless, one cannot totally exclude a contribution from this high-affinity receptor in explaining the increase in VEGF binding in the diabetic kidney.

The increase in VEGF in the diabetic kidney is consistent with in vitro studies suggesting that glucose can induce VEGF expression. Williams et al. (36) have reported that acute hyperglycemia induces VEGF gene expression in vascular smooth-muscle cells, via a protein kinase C (PKC)-dependent pathway. A link between PKC and VEGF is further suggested by studies with an orally active PKCβ inhibitor (37). $PKC\beta_{II}$ inhibition was associated not only with reduced VEGF-induced retinal permeability (37) but also with retardation of development of albuminuria in streptozotocin-induced diabetic rats (38).

The persistent expression of VEGF in the diabetic kidney may relate not only to the effects of hyperglycemia per se but to the effects of advanced glycated end products (AGEs) that accumulate in diabetic tissues over weeks to months (39). These AGEs have been shown in vitro to activate VEGF expression in retinal Müller cells (40) and in vivo to upregulate VEGF expression in the retina, including cells within the ganglion and inner nuclear layers (41). Various other factors relevant to the pathogenesis of diabetic nephropathy have also been shown to promote VEGF expression, including stretch (42), angiotensin II (43), and a number of

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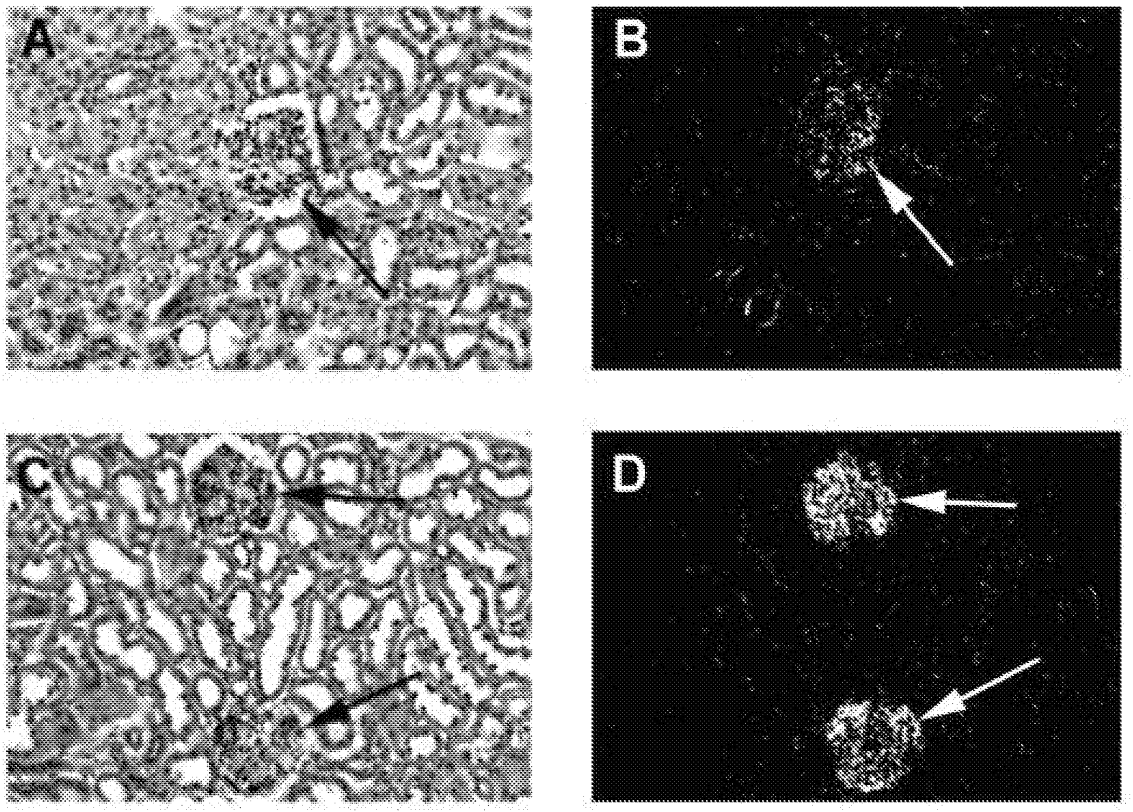


FIG. 10. In vivo binding of ^{125}I-VEGF in the kidney. Light microscopic autoradiographs of kidney cortex from rats administered ^{125}I-VEGF in vivo. Total binding for ^{125}I-VEGF in glomeruli in control (*A*: light field; *B*: dark field) and diabetic (*C*: light field; *D*: dark field) rats. Magnification 200×.

cytokines (44-46). Since it is likely that diabetic nephropathy occurs as a result of an interaction of metabolic factors and hemodynamic factors, it is of particular interest that VEGF expression may be particularly enhanced in the context of angiotensin II and hyperglycemia (47). Transforming growth factor (TGF)-β1 has been reported to downregulate VEGFR-2 expression (48) but to increase gene and protein expression of its ligand, VEGF (49). Since TGF-β1 has been reported to increase in the kidneys from long-term diabetic rats (50,51), it is possible that this cytokine was involved in reducing renal VEGFR-2 overexpression and stimulating VEGF expression in long-term diabetic rats, as was observed in the present study.

The increase in VEGF and VEGFR-2 has been previously reported in the retina in experimental diabetes by several groups (12,13). Hammes et al. (12) reported that there is minimal evidence of expression of VEGF or its receptors in the normal retina with clear evidence of significant synthesis of VEGF and its receptors in the diabetic retina. This was confirmed by Gilbert et al. (13), who also reported that in experimental diabetes there were increased levels of VEGF and VEGFR-2 in the retina. The present study in the kidney provides evidence that the changes observed in the retina are not restricted to one site but also occur in the kidney. From a clinical perspective, a close association has been noted between vision-threatening retinopathy and diabetic renal disease (14,52). This association may be considered to be due to similar pathogenic mechanisms. VEGF must be viewed as a primary candidate for mediating both microvascular complications. The presence of increased ligand as well as increased receptor is suggestive of activation of the VEGF pathway in diabetes. Although it is likely that VEGF plays an important role in ocular neovascularization, its role in the kidney remains to be fully delineated (15). It is clear that VEGF and its receptors are vital for renal development, based on studies in VEGF and VEGF receptor knockout mice (53,54) and on studies involving postnatal administration of neutralizing antibodies to VEGF (55). The localization of VEGF to the glomerular epithelial cell (podocyte) in the current study is similar to the findings of others (56). It has been suggested that VEGF may contribute to the relaxing capacity of the renal vasculature so as to alleviate vascular injury and re-establish vascular integrity (56,57). The importance of this phenomenon in diabetes remains speculative, and, from the present study, it is not possible to determine if overexpression of VEGF in the kidney is deleterious or represents a compensatory mechanism to attenuate renal injury.

The major biological actions of VEGF appear to be the enhancement of vascular permeability and angiogenesis (1). In the retina, these two actions of VEGF are considered rel-

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evant to the development of macular edema and proliferative retinopathy. By contrast, it remains uncertain whether these biological actions of VEGF are relevant to the changes observed in the diabetic kidney. Diabetes is associated with an early increase in albumin permeability, which ultimately leads to the development of albuminuria (58). VEGF is a very potent inducer of permeability (59), and it is possible that this action of VEGF is enhanced in diabetes due to increased synthesis of this peptide within the glomerulus. The importance of VEGF in mediating this effect in diabetes has been suggested by studies in which glucose-induced changes in albumin permeation and blood flow could be prevented by neutralizing antibodies to VEGF (60) or a nonpeptide VEGF antagonist (61).

With respect to VEGF's action as an endothelial mitogen–promoting angiogenesis, the status of the endothelial cell in the diabetic kidney and whether there is associated angiogenesis remains controversial. Østerby et al. (62) have suggested that angiogenesis may occur in human diabetes with duplication of the efferent arteriole. Several other groups have also reported increased blood vessel growth in the diabetic kidney (63–65), but the link of these phenomena to VEGF and VEGF receptor expression remains to be delineated. Indeed, a specific antagonist of VEGF will ultimately be required to determine if VEGF is involved in mediating many of the functional and possibly structural changes observed in diabetic nephropathy.

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VEGF-mediated inflammation precedes angiogenesis in adult brain

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Abstract

Vascular endothelial growth factor (VEGF) has been shown to induce angiogenesis when infused continuously into adult rat brain tissue. In addition, VEGF has been shown to enhance permeability in brain vasculature. Adult rats were continuously infused with mouse VEGF into neocortex for up to 7 days. We studied the development of VEGF-induced vasculature in rat neocortex and evaluated the temporal expression of a wide variety of markers for inflammation and vascular leak in relation to the angiogenic response using immunohistochemistry and Western blot analysis. We report here that VEGF-mediated inflammation in brain is characterized by upregulation of ICAM-1 and the chemokine MIP-1α, as well as a preferential extravasation of monocytes. VEGF causes a dramatic breakdown of the blood–brain barrier, which is characterized by decreased investment of the vasculature with astroglial endfeet. Perivascular cells, in contrast, increase around the newly formed cerebrovasculature. In addition, breakdown of the blood–brain barrier, leukocyte extravasation, and extracellular matrix deposition occur before vascular proliferation. Furthermore, administration of low doses of VEGF induces permeability and inflammation without appreciable vascular proliferation.
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Keywords: Cytokine; Blood–brain barrier; Permeability; Vasculature; Chemokine; VEGF; Inflammation; MIP-1α; Monocytes

Introduction

Vascular endothelial growth factor (VEGF) has been shown to induce vascular proliferation in a variety of tissues (Bauters et al., 1994; Krum et al., 2002; Proescholdt et al., 1999; Rosenstein et al., 1998; Springer et al., 1998; Takeshita et al., 1994), and studies of VEGF null mutant mice have shown that VEGF is necessary for angiogenesis and vasculogenesis in the developing embryo (Ferrara et al., 1996). Consequently, VEGF has been evaluated in clinical trials as a pro-angiogenic factor to treat ischemic conditions (Isner, 1998; Isner et al., 1996; Losordo et al., 1998). Although VEGF's role as an angiogenic factor is well established, it was originally discovered and characterized based on its ability to increase vascular permeability (Senger et al., 1986). VEGF has been shown to increase vascular leak of both proteins and particulates, and it has been proposed that this increase in permeability is a necessary prerequisite for the induction of angiogenesis (for reviews, see Dvorak et al., 1995, 1999).

Many human diseases are characterized by pathological angiogenesis, such as proliferative vascular retinopathies, psoriasis, rheumatoid arthritis, endometriosis, and tumors. In these conditions, VEGF levels are known to be increased before and/or during the angiogenic process (Bhushan et al., 1999; Brown et al., 1993, 1995; Detmar et al., 1994; Harada et al., 1998; Jackson et al., 1997; Kikuchi et al., 1998; Lashkari et al., 2000; Mahnke et al., 2000). In addition, VEGF levels are elevated in many human diseases, or animal models of human disease, which are characterized by inflammation and vascular leak, such as cerebral ischemia, tumor ascites, trauma, early diabetic retinopathy, preeclampsia, ovarian hyperstimulation syndrome, and status epilepticus (Baker et al., 1995; Boulton et al., 1998; Cobbs et al., 1998; Croll et al., 2002; Grad et al., 1998; Hayashi et al., 1997; Issa et al., 1999; Kovacs et al., 1996; Kraft et al.,

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1999; Lee et al., 1999; Lennmyr et al., 1998; Levin et al., 1998; McClure et al., 1994; Neulen et al., 1995; Pichiule et al., 1999; Plate et al., 1999; Sievin et al., 2000; Spirin et al., 1999; Zebrowski et al., 1999). Therefore, a clear understanding of VEGF-mediated angiogenesis and vascular leak is critical if VEGF-related treatments for these pathological conditions are to be evaluated.

We have employed continuous infusion of exogenous VEGF protein into adult rat neocortex as a model for understanding more fully the nature and development of VEGF-induced changes in cerebral blood vessel structure and function. VEGF induces an angiogenic response when infused continuously into adult rat brain via osmotic minipump, or when delivered by way of an adenoviral vector (Proescholdt et al., 1999; Rosenstein et al., 1998). When applied to the brain surface or parenchyma, VEGF induces a rapid and dramatic increase in vascular permeability (Dobrogowska et al., 1998). Our findings show that VEGF acts either directly or indirectly as a potent pro-inflammatory cytokine in adult rat brain, and that increases in vascular permeability and indices of inflammation precede VEGF-induced angiogenesis of brain vasculature.

Materials and methods

Subjects

Adult male Sprague–Dawley rats (300–400 g) from Taconic laboratories (Germantown, NY) were housed two per cage within a temperature and humidity-stabilized animal facility with food and water available ad libitum. Animals were maintained on a 12:12 light–dark cycle (lights on 0600 h) and were allowed to acclimate for at least 1 week before any experimental manipulations. All experiments were conducted under the auspices of protocols approved by the Regeneron Animal Care and Use Committee.

Surgeries

Animals were anesthetized with chloral hydrate (170 mg/kg)–pentobarbital (35.2 mg/kg) ip. Cannulae (2 mm long; Plastics One, Roanoke, VA) were stereotaxically implanted in the right frontoparietal cortex (0.5 mm anterior, 3.2 mm lateral to bregma) as described previously (Croll et al., 1998). Polyvinyl catheters (Bolab, Lake Havasu City, AZ) were attached to the cannulae with cyanoacrylate glue, filled with sterile PBS, and heat-sealed (Loctite 495, Loctite Corp., Newington, CT). One week later (to permit surgical trauma to attenuate), animals were briefly anesthetized with either halothane (5% induction, 1.5% maintenance) in 1:2 oxygen–nitrous oxide or isoflurane (2.5% induction, 2% maintenance) in oxygen. An incision was made in the nape of the neck, the tip of the catheter was cut, and an osmotic minipump (model 2002, 0.5 μl/h, Alza Corporation, Palo Alta, CA) was attached to the catheter using cyanoacrylate glue.

Minipumps containing VEGF were attached to preimplanted PBS-filled catheters on day 0. Because the pumps had to first pump PBS from the catheters into the brains before VEGF protein could reach the brain, VEGF took an average of 21–23 h to reach the brain. Hence, the 1-day time point represents one to several hours of VEGF infusion, rather than a full day of VEGF infusion. Minipumps contained either recombinant mVEGF 164 in PBS, VEGF + Flt-Fc, or PBS (proteins produced at Regeneron). In some experiments, comparison proteins (hFc, hAngiopoietin 1, and hAngiopoietin 2) were infused instead of PBS at concentrations equaling or exceeding the VEGF dose to control for nonspecific protein effects. Angiopoietin 1 and 2 were selected for infusion to control for nonspecific effects of protein growth factors on brain tissue. The hFc protein was selected for infusion both as a general protein control and as a control for the hFc portion of the Flt-Fc. The responses seen in animals infused with control proteins were not different from those observed in animals infused with PBS, except for an occasional tendency toward increased microglial reaction at the infusion site. For dosing experiments, VEGF was given at 30, 60, 120, or 240 ng/day. Flt-Fc, which binds to VEGF with high affinity, was co-infused with either the 120 or 240 ng/day dose of VEGF at 4.8 or 9.6 μg/day (an approximately 10-fold molar excess compared to VEGF). Because Flt-Fc binds to the exogenous VEGF, preventing it from binding to its endogenous receptors, any nonspecific effects of infusion of VEGF protein into brain tissue should be prevented by co-infusion with Flt-Fc. Indeed, nonspecific tissue irritation would be expected to worsen with the addition of increased protein load (i.e., VEGF + Flt-Fc as opposed to just VEGF). Studies of the chronological development of the VEGF-induced vascular responses and detailed analyses of the 7-day neovasculature used 240 ng/day of VEGF. All experiments were repeated at least once, with group sizes of three to six animals in each treatment group in each study, to ensure that the results obtained were consistent.

Immunohistochemistry

After an overdose of chloral hydrate-pentobarbital, animals were exsanguinated and perfused transcardially with heparinized saline (5 IU/l) followed by 4% buffered paraformaldehyde at 4°C. Some animals received a bolus intravenous injection of 0.026-μm-diameter fluorescent microspheres (Duke Scientific, Palo Alto, CA) 30 min before exsanguination and perfusion. Brains were removed and placed in 30% buffered sucrose and later sectioned at 40 μm in the coronal plane. Sections were stored in cryoprotectant (Watson et al., 1986) at −20°C until stained. Brains were immunostained with the antibodies listed in Table 1 using a biotinylated secondary antibody-avidin-peroxidase reaction (Vectastain Elite kit, Vector Laboratories, Burlingame, CA) as described previously (Morse et al., 1993).

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Table 1
Antibodies used for immunostaining

Stains for	Antibody	Species	Dilution	Supplier	City
Vascular endothelial cells	RECA	mouse	1:250	Serotec	Raleigh, NC
Leukocytes	OX-1	mouse	1:10,000	Harlan Bioproducts	Cincinnati, OH
Microglia (macrophage)	OX-42	mouse	1:10,000	Serotec	Raleigh, NC
Astroglia	GFAP	rabbit	1:60,000	Dako	Carpinteris, CA
Immunoglobulin	Rat IgG	rabbit	1:1500	Vector Laboratories	Burlingame, CA
Nitric oxide synthase	eNOS	rabbit	1:250	Transduction Laboratories	Lexington, KT
T cells	R73	mouse	1:5000	Serotec	Raleigh, NC
Monocyte/macrophage	ED-1	mouse	1:15,000	Serotec	Raleigh, NC
ICAM-1	CD-54	mouse	1:10,000	Serotec	Raleigh, NC
Smooth muscle cells/pericytes	α-SMA	mouse	1:500	Dako	Carpinteris, CA
Fibrinogen	Fibrinogen	rabbit	1:20,000	Dako	Carpinteris, CA
VEGF	VEGF	goat	1 μg/ml	R&D Systems	Minneapolis, MN
Proliferating cells	Ki67	mouse	1:100	PharMingen	San Diego, CA
Proliferating cells	BrdU	mouse	1:833	Becton-Dickinson	Franklin Lakes, NJ
Proliferating cells	Histone H3	rabbit	1 μg/ml	Upstate Biotechnology	Lake Placid, NY
Proliferating cells	PCNA	mouse	1:100	Dako	Carpinteris, CA

Quantification

Changes in vascular quality were evaluated quantitatively using stereological techniques. Animals were perfused with 2% paraformaldehyde, the brains were removed, and then frozen in methylbutane on dry ice. Brains were stored at −80°C until sectioned on a cryostat. Within each group, a third of the brains each were pseudorandomly selected for sectioning at 10 μm in coronal, sagittal, and transverse planes. Stereology was performed on RECA-stained slides after acquisition of images by a DAGE MTI CCD725 video camera (Dage, Michigan City, IN) attached to a Nikon microscope (Morrell Instruments, Melville, NY). Images were analyzed using the NIH Image software (NIH, Bethesda, MD). All vasculature within the approximated field of VEGF diffusion was evaluated (a 3-mm square block of cortex centered on the cannula tip).

Percentage of tissue area containing vessels was determined by point-count stereology. An acetate containing points in a grid pattern were overlaid on the images taken from sections sampled every 200 μm throughout the approximately 1.5 mm extent of VEGF diffusion (for a total of approximately seven to eight sections analyzed per animal). All points (intersections) which laid over a vessel were counted and were represented as a proportion of total points. Points were counted if they either lay on a RECA-positive lumen or lay within a space completely encircled by a circumferential RECA-positive lumen. Spaces in the tissue produced by the cannula track were excluded from analysis. Percentage of vascular area for each brain was expressed as percentage of tissue area occupied by vessels averaged over all of the sections. Percentage of tissue area occupied by vasculature was compared for VEGF (n = 7) vs. PBS-infused (n = 7) animals using an independent-groups Student's t test with alpha set at 0.05.

Changes in the proportion of tissue area occupied by vasculature can be attributed either to changes in vascular diameter or changes in vessel length. Vessel diameters were also measured using NIH Image on the same sections sampled for vessel density measurements. Vessels were sampled using a pseudorandomly oriented grid. Vessel diameter was taken by measuring the length of the line representing the smallest perpendicular distance across the vessel. Because more of the grid intersections in the VEGF animals than the PBS animals were overlying vasculature, more vessels were sampled for the VEGF than the PBS animals. However, the mean diameter was determined for each section and then for each animal, such that only one value was statistically analyzed per animal. Vascular diameter was compared for VEGF vs. PBS-infused animals using an independent groups Student's t test with alpha set at 0.05.

All stereological analyses and histological analyses were conducted by observers blind to the treatment condition of the animals from which the tissue was taken.

Western blot analysis

After 7 days of infusion with VEGF or PBS, animals were anesthetized with chloral hydrate-pentobarbital and were rapidly decapitated. The brains were removed and placed inside a 1.5-mm brain block (Zivic-Miller, Allentown, PA). The brain was cut coronally through the cannula track, and the 1.5-mm slab on either side of the track was taken and placed on a cold metal block. Using a fine scalpel blade, cuts were made 1.5 mm lateral and medial to the cannula track in the cortex. The tissue between the cuts in each slab was taken through the entire depth of cortex, placed in a vial, and immediately frozen on dry ice. Tissue was also taken from naive rats.

Tissue was homogenized in lysis buffer containing 1.0% NP40, 0.5% deoxycholic acid, 0.1% SDS, and protease inhibitors (1 Complete tablet, Boehringer Mannheim/Roche, Indianapolis, IN). Lysate (10–20 μl), diluted from 1:1 to 1:5 with sample buffer, was loaded onto each lane of a 12% Tri-Glycine pre-cast Western blot gel. A nonreducing gel was run at 150 V using a Novex Minicell

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II gel electrophoresis unit (Novex/Invitrogen, Carlsbad, CA) for 2 h. Protein was transferred to a nitrocellulose membrane (Novex/Invitrogen) for 2–16 h. After transfer, membranes were washed in TBS-T and transferred into a solution containing primary antibody for all Westerns except for the rat IgG Western analysis (rabbit anti-fibrinogen antibody, 1:1000, Dako, Carpinteris, CA; mouse anti-ICAM-1 antibody, 1:1000, Serotec, Raleigh, NC). After an overnight incubation, membranes were washed in TBS-T and transferred to an HRP-conjugated secondary antibody (for rat IgG: goat anti-rat, 1:10,000, Boehringer Mannheim/Roche; for fibrinogen: goat anti-rabbit, 1,10,000, Chemicon, Temecula, CA; for ICAM-1: goat anti-mouse, 1:5000, Boehringer Mannheim/Roche) for 1 h. After washing, membranes were treated with ECL (Amersham Pharmacia Biotech, Piscataway, NJ) and tranferred onto film (Kodak, Rochester, NY). Films were exposed from 0.5 s to 2 min. For all antibodies, secondary-only gels were run to control for nonspecific bands. In addition, positive protein controls were available and run for fibrinogen (Sigma, St. Louis, MO) and rat IgG (IgG1κ, Serotec).

LightCycler PCR

Tissue was collected for RT-PCR analysis of chemokine levels as described for Western blots, except that equivalent tissue was also taken from the contralateral hemisphere of each animal. Tissues were taken after 2, 4, or 7 days of 240 ng/day VEGF. RNA levels were determined for MCP-1, RANTES, MIP-1α, and GRO-α. Lightcycler PCR was performed as previously described (Lie and Petropoulos, 1998; Ransohoff et al., 2002; Schreiber et al., 2001). Data were analyzed using a 2 (treatment) × 3 (time) × 2 (hemisphere) mixed factorial ANOVA, with alpha = 0.05. Tissue was collected from two separate cohorts of animals, and the data were generated by an experimenter blind to the treatment group of the animals. The control group for the first cohort was vehicle (PBS), and the control group for the second cohort was a protein, human Fc, infused at a dose equivalent to that of VEGF. Data from the two control groups were compared using an ANOVA and were combined for the final analysis after confirming statistically that the results from these groups did not differ. In addition, data from the two cohorts were not found to differ from each other overall by ANOVA.

In situ hybridization

Slides were taken for in situ hybridization from those prepared for the quantitative analysis. The MIP-1α riboprobe was prepared as previously described (Glabinski et al., 1997). In situ hybridization was conducted as previously described (Davis et al., 1996), except that slides were exposed to emulsion for 4 weeks before developing. A control slide from each brain was hybridized to the sense probe, and no specific hybridization pattern was detected.

Results

VEGF dose response

PBS or VEGF (30, 60, 120, or 240 ng/day) were infused continuously into the neocortex of adult male Sprague–Dawley rats for 7 days. Tissue was collected and processed histologically for evaluation of vascular changes. Immunostaining for rat endothelial cell antigen (RECA) revealed that there was little obvious change in cerebral vascular density or morphology when VEGF was infused at a dose of 30 ng/day. At 60 ng/day, small increases in vascular density, tortuosity, and vessel diameter became apparent. These changes became progressively more marked when VEGF was infused at 120 and 240 ng/day (Fig. 1). To confirm the effect of VEGF infusion on vascular leak, tissue sections were also stained for rat IgG, which is excluded from the brain parenchyma of normal adult rats. In rats infused with PBS alone, only a very small amount of rat IgG staining was evident immediately adjacent to the cannula track. However, infusion of VEGF produced a marked extravasation of rat IgG within the brain parenchyma. The amount of rat IgG detected in brain increased in a dose-dependent manner (Fig. 1). Additional sections were immunostained with the pan-leukocyte marker OX-1 to assess infiltration of inflammatory cells into the brain. Few, if any, leukocytes were found in brains infused with PBS, but even the lowest dose of VEGF (30 ng/day), which produced no appreciable changes in vascular morphology, led to a marked extravasation of leukocytes (Fig. 1). The magnitude of the inflammatory response was again clearly dose-dependent. In contrast, infusion of comparison proteins such as human recombinant Angiopoietin-1 and human Fc produced no increase in the extravasation of rat IgG or leukocytes compared to PBS alone (Angiopoietin-1 data shown in Fig. 2C). Furthermore, co-infusion of a 10-fold molar excess of Flt-Fc blocked the leak, inflammation, and increased vasculature induced by VEGF administration (data not shown for leak and inflammation; vasculature shown in Fig. 2D).

Characterization of the VEGF-induced Neovasculature

Vascular morphology

An extensive characterization of the VEGF-induced neovasculature was undertaken following 7 days of infusion. Immunostaining for VEGF revealed that detectable levels of infused protein were apparent within a 1.5-mm radius from the cannula tip (Fig. 3A). RECA immunostaining further revealed that alterations in the normal morphology of brain vasculature were confined to the region of VEGF distribution (refer back to Fig. 2B). The central portion of the infused region was characterized by the presence of a tortuous, large-caliber vascular tangle. At the margin of the VEGF infusion, where VEGF immunostaining was lightest, cerebral vessel morphology was relatively normal,

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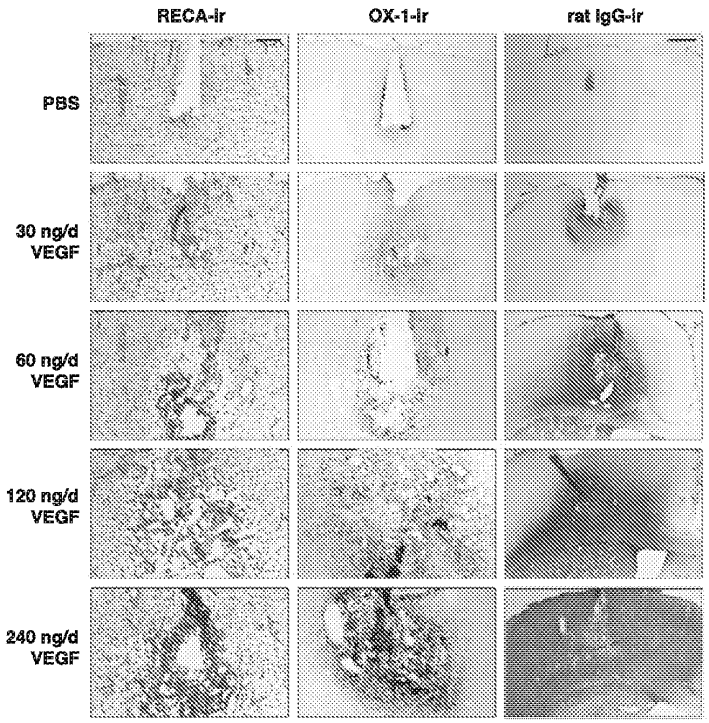


Fig. 1. Immunolocalization of rat endothelial cell antigen (RECA, for visualization of blood vessels), OX-1 (pan-leukocyte), and rat IgG (to visualize extravasated plasma proteins) in brains infused with either PBS or 30, 60, 120, or 240 ng/day of VEGF for 7 days. Angiogenesis, inflammation, and vascular leak were all dose-dependent effects of continuous VEGF infusion. The experiment was conducted twice ($n = 4–6$ per group each time) with comparable results. Scale bar = 300 μm for RECA and OX-1, and 625 μm for rat IgG.

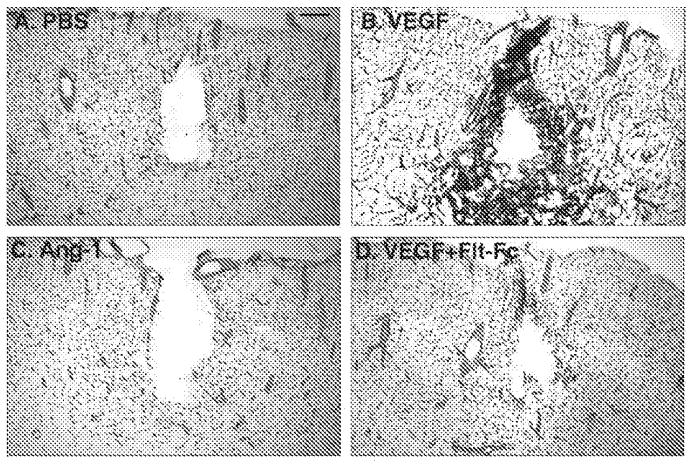


Fig. 2. Immunostaining for RECA after continuous infusion into cortex for 7 days. (A) Infusion of phosphate-buffered saline (PBS) results in minimal vascular change around the cannula track. (B) Infusion of 240 ng/day VEGF induces marked angiogenesis. (C) Infusion of Angiopoietin-1 cannot be differentiated from infusion of PBS. (D) Co-infusion of the receptor body Flt-Fc with VEGF completely prevents VEGF-induced angiogenesis. Each observation was made consistently (in a minimum of two experiments containing four animals per infusion group). Scale bar = 200 μm.

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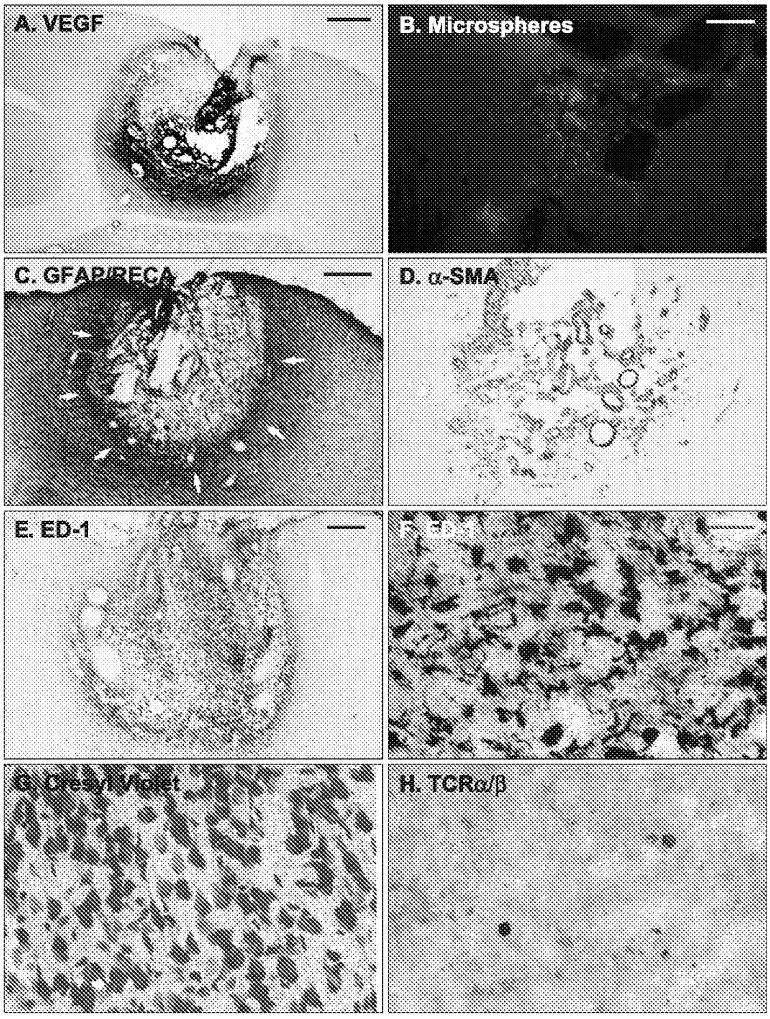


Fig. 3. Cortex infused with 240 ng/day VEGF for 7 days. For panels A–E, the area outside the region of VEGF diffusion displays normal vasculature and can be observed at the edges of the photomicrographs. PBS-infused brains have a similar appearance to the areas outside of the range of VEGF diffusion. (A) VEGF immunostaining shows diffusion of VEGF protein approximately 1.5 mm from the cannula track. (B) Fluorescent microspheres (0.026 μm in diameter) administered intravenously 30 min before sacrifice show that the vasculature within the infused site is acutely leaky at 7 days. (C) A GFAP (dark brown/black) stain shows that there is a dense halo of reactive astroglia located at the peripheral margin of the VEGF-infusion site (approximate boundary with unexposed tissue indicated by the white arrows). In contrast, the center of the VEGF-infusion site is relatively depleted of astrocytes, such that the neovasculature is not well invested with astroglial endfeet. (D) In contrast, α-smooth muscle actin (αSMA) immunostaining illustrates an increased investment of the neovasculature by α-SMA-positive perivascular cells. (E) ED-1, a macrophage/monocyte marker, reveals that most of the inflammatory infiltrate is of monocyte/macrophage origin. (F) A higher magnification view of ED-1-positive cell profiles. (G) Cresyl violet staining after 1 day of VEGF infusion shows the presence of occasional PMNs (example illustrated with an arrow). PMNs are also rarely observed at later time points after VEGF infusions. (H) T cell stain reveals that T cells are not major components of the VEGF-induced immune response. An arrow indicates a TCR-positive cell profile. All findings were confirmed in at least two independent experiments ($n = 4–6$ animals per group per experiment). Scale bars = 500 μm for A, 300 μm for B, 545 μm for C, 340 μm for D and E, and 30 μm for F, G, and H.

though the distance between vascular elements was clearly increased because of local edema. Despite the relative decrease in vascular density at the periphery of the VEGF infusion sites, quantification of vascular density by point-count stereology revealed a significant increase in mean vascular density across the entire field of the infusion in VEGF vs. PBS-treated animals (Table 2). In addition, analysis of vessel diameters revealed a statistically significant increase in mean vessel caliber in the field of VEGF infusion (Table 2).

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Table 2
Quantification of vascular parameters

Measure	Vehicle	VEGF	*t* test results
Vascular density (%)	15.3	36.9	$t(12) = 4.429$, $P < 0.0008$
Vascular diameter (μm)	7.1	17.1	$t(12) = 4.469$, $P < 0.0008$

Each value represents the mean value obtained after quantification of seven animals in each group after 7 days of infusion with 240 ng/day VEGF.

Vascular leak

VEGF-induced neovasculature was characterized by a markedly abnormal vascular permeability, as evidenced by the extravasation of both endogenous serum proteins to demonstrate chronic leak (rat IgG, Fig. 1) and fluorescent microspheres (0.026 μm in diameter), injected intravenously 30 min before sacrifice, to demonstrate acute leak (Fig. 3B). Both the endogenous serum proteins and the microspheres are normally excluded from the brain parenchyma, and there is no specific biological mechanism by which microspheres could be actively transported into the brain during pathological states. Therefore, leakage of microspheres not only demonstrates acute leak at time of sacrifice, but is also likely to reflect a generalized, nonspecific leak.

Astrocytes and perivascular cells

The leak of proteins and particulate matter into the brain suggested that the blood–brain barrier was compromised in the VEGF-affected neovasculature. One important contributor to the blood–brain barrier is astroglial endfeet. GFAP immunostaining revealed a marked decrease in GFAP immunoreactivity within the field of VEGF infusion and intensified staining of reactive glia along the boundary between the affected and unaffected tissue (Fig. 3C). Where VEGF concentrations were highest, the few remaining astroglial endfeet did not closely invest the blood vessels as they normally do in rat brain. Interestingly, immunostaining for α-smooth muscle actin (SMA) showed that the investment of vasculature with SMA-positive perivascular cells was not decreased but, rather, was dramatically increased by VEGF infusion (Fig. 3D).

Inflammation

As previously noted, VEGF infusion produced a marked local inflammatory response. Therefore, additional immunostaining studies were conducted to determine the identity of the leukocytes at the infusion site. ED-1 immunostaining revealed that the majority of the inflammatory cells located within the region of VEGF infusion were of monocyte/macrophage origin (Figs. 3E and F), although very few cells were polymorphonuclear cells (Fig. 3G) and almost none were lymphocytes (TCRα/β stain, Fig. 3H). Because the extravasation of inflammatory cells is typically accompanied by the upregulation of adhesion molecules, which initiate leukocyte rolling and sticking, we evaluated the expression of the adhesion molecule ICAM-1. ICAM-1 was dramatically upregulated in the brains of the VEGF-infused animals (Fig. 4), coincident with both inflammation and the leak of rat IgG and fibrinogen into brain tissue (Fig. 4).

Development of VEGF-induced neovasculature

Vascular morphology

To evaluate the development of VEGF-induced vascular tangles, brains were taken after 1, 3, 5, or 7 days of VEGF infusion. After 1 day of infusion, there was no evidence of VEGF-induced vascular changes (Fig. 5). At 3 days of infusion, changes in vascular morphology were very subtle and variable (Fig. 5). Consistent changes in vascular morphology, including increased vessel diameter and tortuosity, were not clearly evident until 5 days after initiation of the infusion and were more pronounced at 7 days (Fig. 5).

Cellular proliferation

Immunostaining conducted with the proliferation marker Ki67 revealed that, consistent with the results observed with the RECA stain, there was no apparent proliferation of endothelial or perivascular cells after 1 day of infusion (data not shown). At 3 days, there were many Ki67-positive cells present within the field of VEGF diffusion, but few of these were endothelial cells (Fig. 6). Staining for proliferating cell nuclear antigen (PCNA), phosphorylated histone H3, and BrdU (injected at 50 mg ip daily in one experiment) all stained similar non-endothelial cell populations (data not shown). Most stained cells appeared

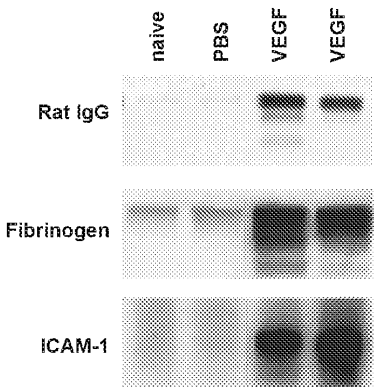


Fig. 4. Unreduced Western blot analyses of rat IgG, fibrinogen, and ICAM-1 found in tissue lysate from rat cortical tissue infused with 240 ng/day VEGF for 7 days. Results obtained from representative unoperated control animals (naive), PBS-infused animals, and two different VEGF-infused animals are illustrated. All Western blot analyses were conducted on two to three different sets of animals at two different protein concentrations (10 and 30 μg). Results were consistent for all proteins analyzed. Results of Westerns were also confirmed by immunostaining tissue sections in separate cohorts of animals (not shown for ICAM-1).

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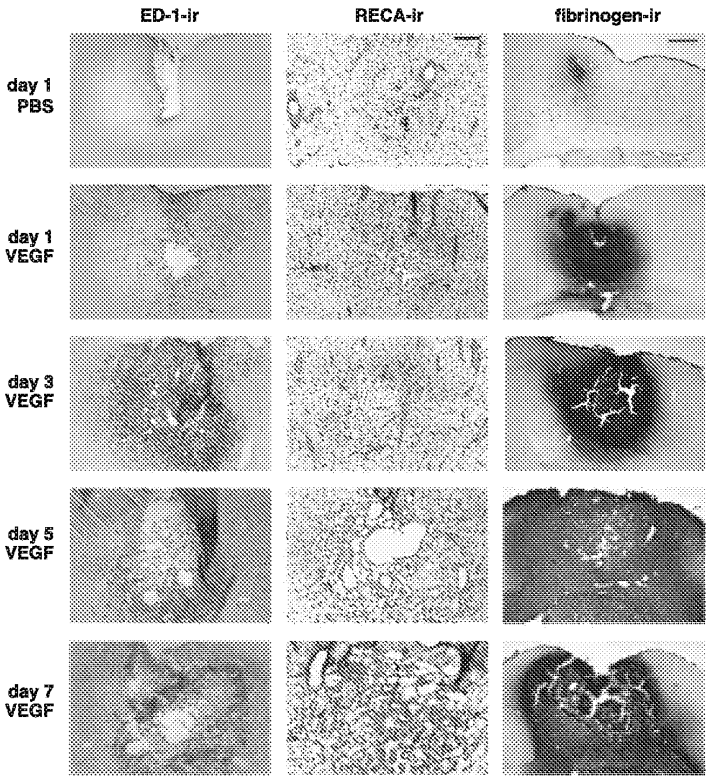


Fig. 5. Immunoreactivity for the monocyte/macrophage marker ED-1, RECA, and fibrinogen are shown for 1, 3, 5, and 7 days after the initiation of 240 ng/day VEGF infusion. Panels in the top row show results after 1 day of PBS infusion. Vascular leak and inflammation are markedly greater after 1 day of VEGF infusions than after 1 day of PBS infusions. Inflammation reaches a plateau by 3–5 days on infusion, and vascular leak plateaus by 5 days of infusions. The experiment was conducted three times ($n = 3–4$ per group per experiment), with each stain completed at least twice. Scale bar = 200 μm for ED-1, 250 μm for RECA, and 625 μm for fibrinogen.

to be of either glial or monocytic morphology (Fig. 6), including dramatic proliferation of both microglia and astroglia at day 7, in agreement with recent findings (astrocytes, Krum et al., 2002). More consistent increases in endothelial cell proliferation were observed at 5 and 7 days of infusion (day 7 shown in Fig. 6). In spite of the dramatic investment of vasculature by smooth muscle cells and pericytes during VEGF infusion, few cells double stained for both Ki67 and α-smooth muscle actin can be observed surrounding the vasculature, suggesting the possibility that these cells were recruited from another site (Fig. 6). Indeed, streams of cells positive for smooth muscle actin, some also positive for Ki67, can be observed in the supracallosal region during VEGF infusion (Fig. 6, 3d Ki67/α-SMA).

Inflammatory cells

In contrast to the delayed effect of VEGF infusion on angiogenesis, OX-1 staining revealed that extravasation of leukocytes began to occur as early as 1 day after the initiation of VEGF infusions (Fig. 5). This inflammation is likely to be a direct consequence of VEGF application because there is no more damage or tissue disruption in VEGF-infused animals than in vehicle or protein-infused animals at early time points (determined by Nissl staining, data not shown). Hematoxylin and eosin staining revealed that polymorphonuclear cells (PMNs) were present, but in very small numbers (refer back to Fig. 3G). T cells, as revealed by TCRα/β immunostaining, were very rarely observed. OX-1 immunostaining revealed, however, that the inflammatory response was marked at day 1, increased dramatically at day 3, and continued at high levels at days 5 and 7 of infusion (Fig. 5). The majority of the inflammatory cells at all time points evaluated were determined to be of monocyte/macrophage lineage based on ED1 immunostaining (compare the monocyte marker ED-1 in Fig. 5 to the pan-leukocyte marker OX-1 in Fig. 1).

Vascular leak

In agreement with the VEGF dose-response results, the temporal development of vascular leak paralleled the time

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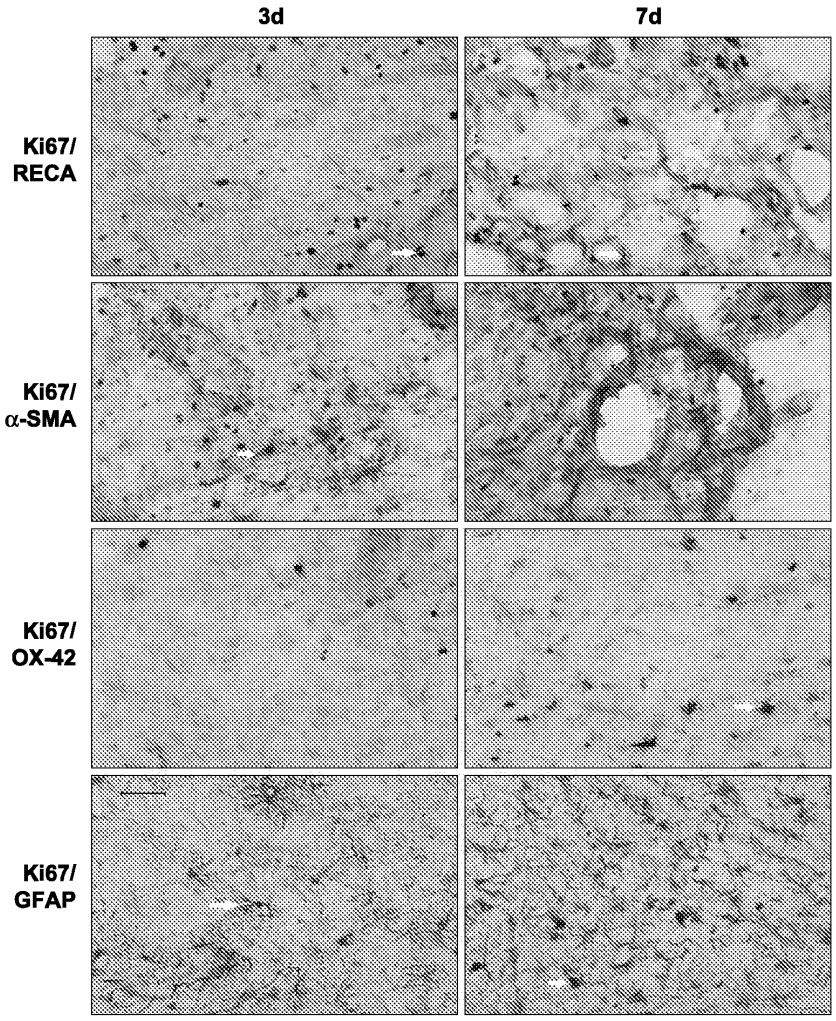


Fig. 6. Double immunoreactivity for Ki67 (a marker of proliferating cells), with RECA, α-SMA, OX-42, and GFAP show that VEGF results, either directly or indirectly, in the proliferation of multiple cell types. Data are shown at 3 and 7 days after the initiation of 240 ng/day VEGF infusion. Ki67 staining was not dramatic at 1 day of VEGF infusions (data not shown). RECA immunostaining reveals that although there are some candidate proliferating endothelial cells (examples shown by arrows), most proliferating cells, especially at 3 days of infusions, are not endothelial cells. In addition, smooth muscle cells did not account for the majority of proliferating cells, although an occasional proliferating smooth muscle actin-positive cell could be identified migrating toward the VEGF infusion site (see arrow). Most proliferating cells were OX-42-positive. Most of these double-positive cells appeared to be monocytic, but at 7 days of infusion, many proliferating cells with a microglial morphology could be identified (see arrow). Large numbers of GFAP-positive cells were double-stained for Ki67. These cells could be first observed at 3 days of VEGF infusions, but were even more numerous at 7 days of VEGF infusions (see arrows). Scale bar = 50 μm.

course of leukocyte extravasation. That is, infusion of VEGF resulted in an influx of plasma proteins and leukocytes into brain parenchyma which preceded its effects on endothelial cell proliferation. A modest extravasation of plasma proteins and leukocytes was already apparent after 1 day of infusion, and dramatic leak and inflammation were consistently evident at 3, 5, and 7 days of infusion (Fig. 5, fibrinogen staining).

Chemokines

Chemokine mRNA levels were evaluated in brains infused for 2, 4, or 7 days with VEGF, PBS, or control protein (human Fc). LightCycler RT-PCR revealed dramatic increases in MCP-1 mRNA [$F(1,94) = 12.116$, $P < 0.0008$] and modest increases in RANTES mRNA [$F(1,94) = 9.049$, $P < 0.004$] in the infused cortices of both control and VEGF-infused brains, but upregulation of

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these chemokines was not significantly associated with infusion of VEGF [$F(1,94) = 1.264$, $P > 0.26$ for MCP-1, $F(1,94) = 2.679$, $P > 0.10$ for RANTES, and additionally, $F(1,44) = 0.529$, $P > 0.47$ for GRO-α, which showed no upregulation in ipsilateral cortex]. In marked contrast, VEGF infusion produced a dramatic and selective upregulation of transcripts for MIP-1α [$F(1,68) = 8.959$, $P < 0.004$; Fig. 7D].

In situ hybridization for MIP-1α mRNA revealed that only a few cells expressing MIP-1α mRNA were evident in PBS-infused brains, and these were located immediately adjacent to the cannula track (data not shown). In marked contrast, in VEGF-treated animals, MIP-1α mRNA was expressed at high levels in many cells located preferentially near the periphery of the field of VEGF infusion (Fig. 7A). In contrast, most extravasated inflammatory cells are located at the core of the infusion (Fig. 7B). The cell bodies of the hybridized cells appeared dark and angular in the thionin counterstain, properties most typically found in microglia, but also sometimes observed in astroglia, pericytes, or other cell types (Fig. 7C).

Discussion

VEGF is a known mediator of angiogenesis and vascular permeability. Our results demonstrate that VEGF also acts, either directly or indirectly, as a potent pro-inflammatory cytokine. In addition, we show that vascular leak and inflammation precede the endothelial cell proliferation that occurs in response to VEGF.

Chronology and dose response of VEGF-induced vascular changes

Vascular changes with VEGF infusion into brain parenchyma occur with a significant delay upon exposure of normal brain tissues to even high levels of exogenous VEGF. This observation also appears to be the case when angiogenesis is initiated by endogenous VEGF. For instance, after cerebral ischemia, VEGF mRNA is increased within hours of the ischemic injury (Cobbs et al., 1998; Hayashi et al., 1997; Issa et al., 1999, Kovacs et al., 1996; Lee et al., 1999; Lemmyr et al., 1998; Pichiule et al., 1999, Plate et al., 1999), but the angiogenic response

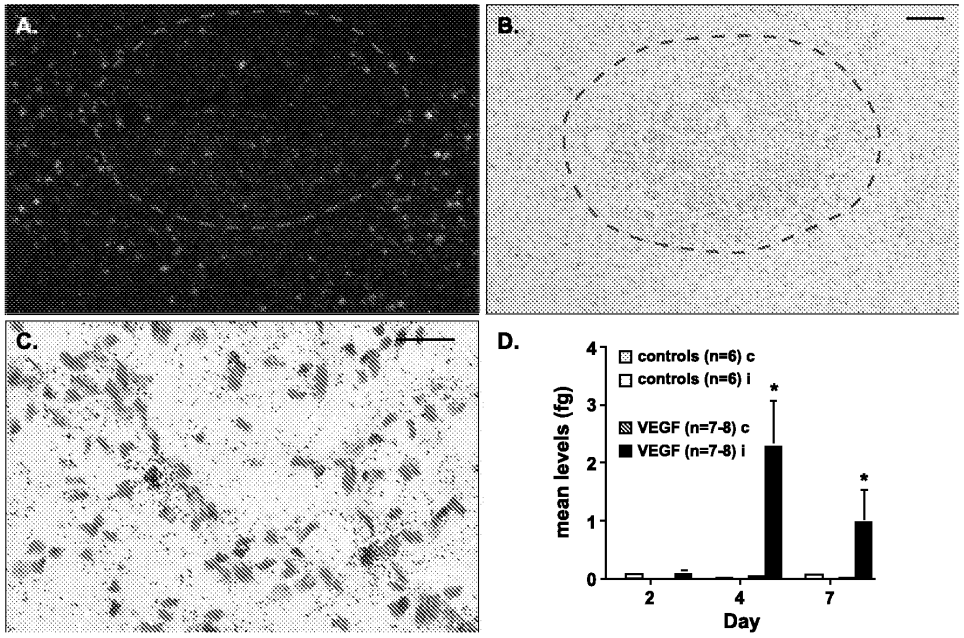


Fig. 7. MIP-1α mRNAs after VEGF infusion. (A) Dark-field photomicrograph of MIP-1α in situ hybridization after 7 days of VEGF infusion. The majority of MIP-1α positive cells lie at the periphery of the infusion site; outlined by the dashed line in panels A and B. Only one or two positive cells were found adjacent to the cannula track in PBS-infused animals. (B) Bright-field photomicrograph from the same section as panel A showing that the majority of the extravasated inflammatory cells were in the core of the VEGF-infused region in which cells hybridized for MIP-1α were sparse. Scale bar for A and B = 200 μm. (C) Higher magnification view of area indicated in panel B showing that MIP-1α positive cells (examples indicated by arrows) have an angular morphology. Scale bar = 50 μm. (D) MIP-1α mRNA levels determined by LightCycler PCR after 2, 4, or 7 days infusion of 240 ng/day VEGF. Results were obtained from two separate cohorts of animals, as described in Materials and methods. c = contralateral side, i = ipsilateral side, $^*P < 0.05$, significantly different from control group.

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is not evident until days later (Beck et al., 2000; Marti et al., 2000). Our observation of delayed angiogenesis was made based on data obtained with RECA immunostaining and proliferation markers. RECA will stain all mature vasculature and most vascular sprouts; however, we cannot rule out the possibility that earlier, RECA-negative basal lamina could have been present before the 3-day time point. Future studies might investigate very early events in the development of vascular sprouts in mature brain vasculature.

In contrast to the apparently delayed angiogenic response, blood–brain barrier compromise occurs very rapidly upon exposure to VEGF. We have observed vascular leak 1 day after VEGF minipump implantation, the earliest time point evaluated, but others have documented vascular permeability within 30 min of acute application (Dobrogowska et al., 1998). In addition, we showed that low doses of VEGF, which did not lead to appreciable angiogenesis within our 7-day infusions, rapidly and substantially increased vascular permeability. VEGF-mediated vascular leak was always accompanied by leukocyte extravasation. Hence, leukocyte extravasation, like vascular leak, occurred at earlier time points and lower doses than angiogenesis.

It has been proposed that VEGF-mediated increases in vascular permeability represent the first step in the angiogenic process (Dvorak et al., 1995, 1999). Vascular leak leads to the deposition of plasma proteins, such as fibrinogen, which can form an extracellular matrix on which endothelial cells can migrate. In this schema, vascular leak facilitates subsequent angiogenesis and may be a necessary pre-requisite for vascular proliferation.

VEGF and inflammation

Although VEGF's roles as a permeability and angiogenic factor are well established, less is understood about VEGF's role in the induction or augmentation of inflammation. We observed a reliable and dramatic induction of a leukocyte infiltrate at early time points of VEGF administration, before the development of obvious angiogenesis. In addition, we observed inflammation at low doses of VEGF insufficient to induce angiogenesis. Using immunostaining, we have shown that the majority of extravasated inflammatory cells are of monocyte/macrophage lineage. VEGF is a monocyte attractant in vitro (Heil et al., 2000; Waltenberger et al., 2000) and stimulates hematopoiesis in vivo (Hattori et al., 2001). The effect of VEGF on monocytes could be direct because monocytes express VEGFR1 (Flt-1) receptors (Sawano et al., 2001).

Consistent with the increased extravasation of leukocytes, we have detected an increase in the adhesion molecule ICAM-1 in the VEGF-infused cortex. VEGF has previously been shown to increase ICAM-1 both in vitro (Kim et al., 2001; Melder et al., 1996) and in vivo (Miyamoto et al., 2000; Proescholdt et al., 1999). ICAM-1 is normally expressed at negligible levels in adult rat brain (refer to naive band in Fig. 4). The increases in this adhesion protein in VEGF-infused brains are consistent with an increased inflammatory response because adhesion molecules encourage leukocyte sticking and rolling in the brain vasculature (for review, see del Zoppo, 1997). VEGF also increases integrin expression on leukocytes (Byzova et al., 2000; Heil et al., 2000). The interaction of leukocyte integrins with ICAM-1 appears necessary for the transendothelial migration of monocytes in response to VEGF (Heil et al., 2000).

Once rolling, leukocytes extravasate into brain in the presence of a leukocyte chemoattractant protein. Among the most common leukocyte attractants are chemokines (for review, see Ransohoff and Tani, 1998). We have discovered that VEGF potently and selectively upregulates the beta chemokine MIP-1α, which attracts or modulates the function of multiple types of inflammatory cells (Alam et al., 1992; Fahey et al., 1992; Schall et al., 1993). VEGF's effect on MIP-1α does not generalize broadly to other chemokines, as VEGF did not significantly upregulate MCP-1, RANTES, or GRO-α. Trauma alone dramatically upregulated MCP-1, as previously reported (McTigue et al., 1998; Sun et al., 2000).

We have yet to determine whether VEGF's effects on the inflammatory chemokine MIP-1α are direct or indirect, although an indirect effect seems likely given the delayed nature of the upregulation. Extravasated monocytes release many cytokines which could act on resident brain cells or newly extravasated immune cells to increase MIP-1α. MIP-1α levels increase in microglia and astrocytes upon exposure to cytokines or ischemia (McManus et al., 1998; Peterson et al., 1997; Takami et al., 1997). The increased expression of MIP-1α in cells surrounding the VEGF infusion site could serve to further propagate the inflammatory response initiated by VEGF infusion.

MIP-1α is increased in neurological disorders associated with vascular permeability and inflammation. For example, MIP-1α is elevated both in human multiple sclerosis lesions (Baranzini et al., 2000; Boven et al., 2000) and in the lesions observed in the animal model, experimental allergic/autoimmune encephalitis (Fischer et al., 2000; Glabinski et al., 1998; Nygardas et al., 2000). In fact, the severity of symptoms in experimental allergic encephalitis is decreased in animals deficient in an MIP-1α receptor, CCR1 (Rottman et al., 2000), although this decreased severity has not yet been observed in MIP-1α deficient animals (Tran et al., 2000). MIP-1α also increases within 4–6 h after cerebral ischemia (Gourmala et al., 1999; Kim et al., 1995; Pang et al., 2002; Spleiss et al., 1998; Takami et al., 1997), concurrent with increased post-ischemic VEGF mRNA levels (Cobbs et al., 1998; Hayashi et al., 1997; Issa et al., 1999; Kovacs et al., 1996;

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Lee et al., 1999; Lemmyr et al., 1998; Pichiule et al., 1999, Plate et al., 1999).

Examples of VEGF-induced inflammation in vivo have been reported previously, albeit sparsely, in the literature. Mice overexpressing endogenous VEGF exhibit marked inflammation in their skins (Detmar et al., 1998; Xia et al., 2003). VEGF delivered via adenovirus induces a monocyte/macrophage infiltrate in brain similar to the inflammatory response which we have observed (Proescholdt et al., 1999). This inflammatory response could not be induced using other adenoviruses, but could be induced using VEGF protein co-infused with viral particles. Here, we demonstrate that administration of VEGF alone is sufficient to induce an inflammatory reaction. Given the complete inhibition of VEGF's actions by Flt-Fc, the potential for this and similar reagents to inhibit not only angiogenesis, but also VEGF-induced inflammation, should be more fully explored.

Our understanding of the role of VEGF-mediated inflammation in the angiogenic process is still developing. However, increasing evidence suggests that, at least in the adult, VEGF-mediated inflammation is inseparable from, and might participate directly in, the initiation of the angiogenic process as has been proposed previously for vascular leak (Dvorak et al., 1995, 1999). Pathological retinal angiogenesis, which can be suppressed by blockade of VEGF-164, has been shown to be inhibited both by monocyte depletion (Ishida et al., 2003) and by deletion of the gene for ICAM-1 (Sakurai et al., 2003). In addition, placental growth factor (PlGF), a VEGF family member which acts at VEGFR1 but not VEGFR2, induces angiogenesis which is blocked after monocyte depletion in animals (Pipp et al., 2003), suggesting the possibility that angiogenesis mediated by VEGFR1 may be critically dependent on monocytes.

Monocytes secrete both degradative enzymes and a variety of trophic and regulatory factors which establish conditions conducive to endothelial cell proliferation and migration (Pakala et al., 2002). Proliferating vasculature expresses high levels of ICAM-1 coincident with increased numbers of adherent monocytes (Scholz et al., 2000). Monocytes, in particular, are frequently associated with proliferating blood vessels (Arras et al., 1998; Schaper and Buschmann, 1999; Scholz et al., 2000) and can open passages in existing vasculature to encourage the development of vascular sprouts (Molodovan et al., 2000). Furthermore, monocytes can differentiate into endothelial-like phenotypes in vitro, especially when treated with VEGF (Fernandez Pujol et al., 2000), raising the possibility that monocytes might contribute directly to angiogenesis as a source of new endothelial cells. Circulating blood cells have been shown previously to integrate into vascular endothelium (Shi et al., 1998). Thus, although the exact role(s) of inflammatory cells in pathological VEGF-mediated angiogenesis remains to be determined, a contributory role seems clear.

Acknowledgments

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Antibodies against Vascular Endothelial Growth Factor Improve Early Renal Dysfunction in Experimental Diabetes

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Abstract. Vascular endothelial growth factor (VEGF) is a cytokine that potently stimulates angiogenesis, microvascular hyperpermeability, and endothelium-dependent vasodilation, effects that are largely mediated by endothelial nitric oxide synthase (eNOS). The expression of VEGF is pronounced in glomerular visceral epithelial cells, but its function in renal physiology and pathophysiology is unknown. VEGF expression is upregulated by high ambient glucose concentrations in several cell types *in vitro* and in glomeruli of diabetic rats. To assess the role of VEGF in the pathophysiology of early renal dysfunction in diabetes, monoclonal anti-VEGF antibodies (Ab) were administered to control and streptozotocin-induced diabetic rats for 6 wk after induction of diabetes. Based on *in vitro* binding studies, an adequate serum VEGF inhibitory activity was achieved during the entire course of anti-VEGF Ab administration. Anti-VEGF Ab treatment but not administration of isotype-matched control Ab decreased hyperfiltration, albuminuria, and glomerular hypertrophy in diabetic rats. VEGF blockade also prevented the upregulation of eNOS expression in glomerular capillary endothelial cells of diabetic rats. Antagonism of VEGF had no effect on GFR and glomerular volume in control rats. These results identify VEGF as a pathogenetic link between hyperglycemia and early renal dysfunction in diabetes. Targeting VEGF may prove useful as a therapeutic strategy for the treatment of early diabetic nephropathy.

It is well established that hyperglycemia is a major risk factor for the development and progression of diabetic nephropathy. Hyperglycemia induces multiple cellular and molecular alterations that presage the development of renal vascular dysfunction. However, the exact sequence of events from exposure to high glucose concentrations to development of renal damage remains equivocal. Early alterations in the diabetic kidney include the development of glomerular hyperfiltration and glomerular hypertrophy, followed by thickening of the glomerular basement membrane, mesangial matrix accumulation, increased urinary albumin excretion rate (UAER), and ultimately progression to glomerular sclerosis. There is compelling evidence for the involvement of various growth factors, including insulin-like growth factor-I (IGF-I) and transforming growth factor-β (TGF-β), in the early renal changes in diabetes (1,2).

Vascular endothelial growth factor (VEGF) is a member of a family of heparin-binding growth factors that very potently stimulate endothelial cell proliferation and play a pivotal role in physiologic and pathologic angiogenesis (3). In addition, VEGF is one of the most potent vascular permeabilizing agents known and has the capacity to induce endothelium-dependent vasodilation. Several lines of evidence indicate that endothelium-derived nitric oxide (NO) acts as a downstream mediator for VEGF (4,5). In cultured endothelial cells, VEGF stimulates endothelial NO synthase (eNOS) expression and activity, resulting in enhanced generation of bioactive NO (6). In the normal kidney, VEGF is strongly expressed in podocytes and its binding sites are localized mainly on glomerular endothelial cells (7–9). Because of this strategic anatomic location, it has been speculated that VEGF may play a role in the regulation of glomerular permeability and glomerular endothelial cell growth (10). Recent studies have demonstrated an upregulation of VEGF and its receptors in kidneys of diabetic rats (11,12). However, whether VEGF plays a causative role in the pathophysiology of diabetic nephropathy remains unknown. Nevertheless, in view of its known biologic properties, its high glomerular expression in physiologic circumstances, and its upregulation in diabetes, VEGF is an ideal candidate to provide a mechanistic link between hyperglycemia and glomerular hypertrophy, glomerular hyperfiltration, and increased glomerular permeability for macromolecules. We therefore investigated the possibility that inhibition of VEGF activity prevents the onset of early renal dysfunction in experimental diabetes. In addition, the effect of VEGF blockade on glomerular eNOS expression was studied.

Materials and Methods

Laboratory Animals

The studies were performed with female Wistar rats (Iffa Credo, Brussels, Belgium) that received care in accordance with the national

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guidelines for animal protection. Diabetes was induced by intravenous injection of streptozotocin (65 mg/kg; Pfanstiel Europe LTD, Davenham, UK). Slow-release insulin implants (Linshin, Scarborough, Canada), with a release rate of 1 U/24 h, were used to maintain moderate hyperglycemia. Age-matched control rats received an intravenous injection of buffer solution and placebo implants composed of palmitic acid. Final experiments were carried out 6 wk after the induction of diabetes. At the beginning of each experiment, plasma samples were drawn for analysis of glucose, fructosamine, and total protein levels.

Anti-VEGF Antibody Treatment

Monoclonal anti-VEGF antibodies (Ab) and isotype-matched control Ab were prepared as described previously (13). Briefly, female 8-wk-old BALB/c mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were immunized, then received a booster three times, 21 d apart, by intraperitoneal and subcutaneous injections of 50 μg of rh-$VEGF_{165}$, emulsified with an equal volume of Complete Freund's Adjuvant for the primary immunization and Incomplete Freund's Adjuvant for secondary immunizations. The mouse with the highest serum titer to rh$VEGF_{165}$ as measured by enzyme-linked immunosorbent assay received an intravenous injection of an additional 30 μg of immunogen in phosphate-buffered saline (PBS), 21 d after the last immunization. Three d later, spleen cells were harvested for production of hybridomas to rh$VEGF_{165}$. Two hybridoma cell lines with highest Ab titer and neutralizing Ab activity were cloned three to four times by limiting dilution in 96-well microtiter plates. Ascites fluid was collected from pristane (Sigma Chemical Co., St. Louis, MO) primed BALB/c mice that received intraperitoneal injections of each of the cloned hybridomas (10^7 cells), and purified IgG was prepared by Protein A chromatography (Sigma). The isotype and light chain composition of the Ab and the characterization of neutralizing activity were performed as described previously (13).

Diabetic rats were either untreated (n = 11) or treated with anti-VEGF Ab (n = 12) or with isotype-matched control Ab (n = 9). One mg of the appropriate Ab was injected intraperitoneally three times per week, starting 2 d after the streptozotocin injection until the final experiments. Age-matched control rats were either untreated (n = 12) or treated with anti-VEGF Ab (n = 7) during 6 wk.

VEGF Binding Studies

To determine the efficiency of the anti-VEGF treatment, we obtained serum samples at baseline and after 2, 4, and 6 wk of treatment with anti-VEGF or control Ab. The samples were drawn from the tail vein just before the next Ab administration. Binding studies were performed using a fusion protein composed of the seven loop ectodomain of Flt-1 fused to the heavy chains of a mouse IgG_{2a} Ab. The fusion protein was captured onto Immulon 4 strip wells with IgG_{2a}-specific goat anti-mouse Ab (Sigma). Increasing concentrations of monoclonal anti-VEGF Ab (used as standards) and increasing dilutions of serum (1:50, 1:100, and 1:250) were incubated separately with 4 ng/ml ^{125}I-VEGF (Biomedical Technologies Inc., Stoughton, MA) for 30 min at 37°C before adding to the receptor. Nonspecific binding was defined as the binding measured in the presence of a 100-fold molar excess of unlabeled VEGF. Incubations were terminated after 60 min at 22°C by washing the wells once with 400 μl of ice-cold PBS, then twice more with 200 μl of ice-cold PBS. Bound VEGF was measured in a gamma spectrometer (Life Technologies, Inc., Schaumburg, IL).

Measurement of UAER and GFR

For determination of UAER, rats were housed in metabolic cages two times for 24 h and the urine was collected. Urine samples were stored at −20°C until analysis. Albumin was determined with an enzyme-linked immunosorbent assay kit specific for rat albumin (Nephrat, Exocell, Philadelphia, PA). To obtain an estimate of GFR, we measured inulin clearance. The rats were anesthetized with thiobutabarbital (Inactin, RBI, Natick, MA; 100 mg/kg intraperitoneally). The trachea was intubated, a jugular vein was cannulated for continuous infusion of isotonic saline at a rate matching diuresis, and a carotid artery was cannulated for drawing of blood samples. Inulin clearance was measured using the slope technique, as described previously (14). A single dose of FITC-Inulin (Sigma, 80 mg/kg) was administered intravenously as a bolus, and plasma samples were obtained at t = 3, 30, 120, 140, 160, and 180 min. FITC-inulin plasma levels were measured with a scanning fluorescence detector (Waters 474, Milford, MA). Inulin clearance was calculated as the ratio of administered dose and area under the curve of the inulin plasma levels.

Measurement of Kidney Weight and Estimation of Glomerular Volume

Kidneys of six animals from each experimental group were rinsed with PBS by retrograde aortic perfusion for 1 min. The right kidney was perfusion-fixed with 2% glutaraldehyde in PBS, stored in the same fixative for 24 h, and rinsed in PBS. Kidneys were embedded in paraplast and stained with Masson-Goldner's trichrome technique. Morphometric analysis was carried out with a semiautomatic image analysis system (VIDS IV, AlTectron, Düsseldorf, Germany) connected to a Zeiss photomicroscope. Cross-sectional glomerular tuft area (A_T, minimal convex polygon) was determined from the mean of 80 random glomerular profiles per animal. Mean glomerular tuft volume (V_T) was calculated as $V_T = \beta/k \times (A_T)(3/2)$, with $\beta = 1.38$, the shape coefficient for spheres and $k = 1.1$, a size distribution coefficient (15).

Immunocytochemistry for eNOS

The expression of eNOS in the glomeruli was investigated using indirect immunocytochemistry. After retrograde aortic perfusion with PBS for 1 min, the left kidney of six animals from each experimental group was removed and snap-frozen in melting isopentane cooled by liquid nitrogen. Five-μm frozen sections were prepared with a 2800 Frigocut E cryostat microtome (Reichert-Jung GmbH, Nussloch, Germany) and fixed in acetone for 10 min. Nonspecific Ab staining was blocked by incubation with PBS containing 0.2% cold-water fish gelatin (Sigma), 2% bovine serum albumin (Sigma), and 2% fetal calf serum (Sigma) for 30 min at room temperature. Incubation with a mouse monoclonal Ab against human eNOS isotype IgG1 (Transduction Laboratories, Lexington, KY), diluted 1:1000 in PBS containing fish gelatin, was carried out overnight at 4°C. Ab binding was visualized with Cy3-conjugated goat anti-mouse IgG Ab (Jackson Laboratories, West Grove, PA). Sections were mounted and viewed with a Polyvar fluorescence microscope (Reichert-Jung). The specificity of the immunolabeling was confirmed by incubation without primary Ab and by incubation with nonspecific Ab.

The intensity of the eNOS Ab staining in the kidneys was evaluated by two independent investigators who were unaware of the status of the animals. Because the receptors for VEGF are localized mainly on glomerular capillary endothelial cells (7–9), eNOS staining was assessed semiquantitatively in these cells. For each kidney, 20 glomerular profiles, cut in equatorial section planes and successively appear-

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ing in the visual field of the microscope when moving the section through the entire depth of the cortex, were evaluated. The number of capillary profiles stained by eNOS Ab was determined and normalized for glomerular surface area.

Immunocytochemical Localization of the Anti-VEGF and Control Ab

To exclude the possibility of precipitation of the Ab or Ab-antigen complexes in the glomeruli, we analyzed a separate group of kidneys (3 diabetes + anti-VEGF Ab, 1 control + anti-VEGF Ab, 2 diabetes + control Ab, 2 diabetes, 2 control). Kidneys were rinsed with PBS by retrograde aortic perfusion for 1 min, then perfusion-fixed with 2% paraformaldehyde and stored in the same fixative for 24 h before being washed with PBS and embedded in paraffin. To detect the murine anti-VEGF Ab, we deparaffinized 5-μm kidney sections with xylene followed by 100% ethanol, rehydrated them with graded ethanol (100%, 95%, and 70%) followed by PBS, blocked them for 1 h at room temperature using 2% normal goat serum in PBS (Sigma), then incubated them for 1 h at room temperature with anti-mouse IgG, Cy3 conjugate (Sigma), diluted 1:500 in PBS containing 2% normal goat serum.

Statistical Analyses

The data are presented as mean ± SEM. ANOVA and unpaired *t* tests were used as appropriate to test statistical significance. The significance level was set at $P < 0.05$.

Results

Characteristics of Laboratory Animals

Diabetic animals had significantly higher plasma glucose and fructosamine levels as compared with the age-matched control rats (Table 1). There were no differences in metabolic control between the Ab-treated and the untreated diabetic rats. Body weights were significantly lower in diabetic rats as compared with control rats. There were no significant differences in body weight among the diabetic groups. Kidney weight was significantly higher in all diabetic animals as compared with the control groups. The kidney weight in the diabetic animals that were treated with anti-VEGF Ab tended to be lower than in the other diabetic groups, but the difference was not significant (Table 1). Food consumption was approximately 70% higher in diabetic animals as compared with controls, without significant differences between the diabetic groups (Table 1).

VEGF Binding Studies

VEGF inhibitory activities were measured after dilution of the serum at 1:50, 1:100, and 1:250. The results of the 1:50 dilution are displayed in Figure 1. At baseline, VEGF inhibitory activity was low and not different between experimental groups. A significantly increased serum inhibitory activity was found at 2, 4, and 6 wk of treatment with anti-VEGF Ab in both diabetic and control rats. At 4 wk, inhibitory activity was somewhat lower in anti-VEGF–treated control rats as compared with anti-VEGF–treated diabetic rats. Because the inhibitory activity of undiluted serum of anti-VEGF–treated rats is almost 100%, the clinical relevance of this difference may be minimal. No changes in VEGF inhibitory activity were seen in diabetic rats during treatment with control Ab, except at 6 wk, when a lower inhibitory activity was noted. The inhibitory activities at 1:100 and 1:250 dilution of the serum were lower than those at 1:50 dilution but followed a similar pattern (data not shown).

Measurement of UAER and GFR

Untreated diabetic rats showed a marked elevation of the UAER after 6 wk of diabetes, which was partially reduced by anti-VEGF treatment but not by administration of control Ab (Figure 2). Inulin clearance was elevated in diabetic rats, as compared with control rats (Figure 3). The development of hyperfiltration in the diabetic rats was largely prevented by treatment with anti-VEGF Ab but not by injection of control Ab. The anti-VEGF Ab treatment did not significantly decrease GFR in control rats.

Glomerular Volume

Glomerular volume was significantly higher in diabetic rats as compared with age-matched control rats. Anti-VEGF Ab treatment decreased glomerular volume in diabetic rats but not in control animals (Figure 4). Administration of control Ab did not affect glomerular volume in diabetic rats.

Immunocytochemical Study of eNOS Expression

Positive eNOS staining was detectable in the endothelial cells of preglomerular vessels (arcuate arteries, interlobular arteries, afferent arterioles), of glomerular capillaries, and of postglomerular vessels (efferent arterioles, peritubular capillar-

Table 1. Gravimetric and biochemical characteristics of the experimental groups[a]

	Control (n = 12)	Control + Anti-VEGF Ab (n = 7)	Diabetes (n = 11)	Diabetes + Anti-VEGF Ab (n = 12)	Diabetes + Control Ab (n = 9)
Glycemia (mg/dl)	144 ± 12	117 ± 8	430 ± 36[b]	438 ± 63[b]	459 ± 31[b]
Fructosamine (μmol/g total protein)	1.78 ± 0.04	1.79 ± 0.06	4.00 ± 0.23[b]	4.17 ± 0.31[b]	3.92 ± 0.28[b]
Body weight (g)	285 ± 4	273 ± 5	253 ± 6[b]	259 ± 8[b]	254 ± 7[b]
Kidney weight (g)	0.91 ± 0.03	0.88 ± 0.02	1.20 ± 0.05[b]	1.12 ± 0.03[b]	1.20 ± 0.05[b]
Food consumption (g)	14.0 ± 1.4	14.1 ± 1.3	24.5 ± 1.5[b]	22.5 ± 1.9[b]	23.5 ± 1.4[b]

[a] VEGF, vascular endothelial growth factor; Ab, antibody.
[b] $P < 0.001$ *versus* controls.

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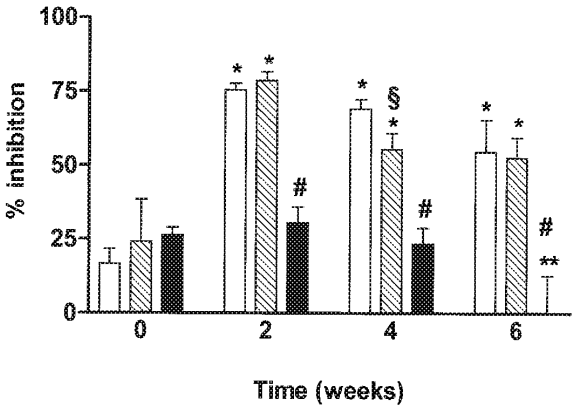


Figure 1. Serum inhibitory activities in diabetic rats + anti-vascular endothelial growth factor (VEGF) antibody (Ab) ($n = 12$, □), control rats + anti-VEGF Ab ($n = 7$, ▨) and diabetic rats + control Ab ($n = 9$, ■) before and 2, 4, and 6 wk after the start of treatment. Inhibitory activities are expressed as percentage of inhibition by 1:50 diluted serum of the binding between ^{125}I-VEGF and Flt-1 *in vitro*. Comparison among time points: *, $P < 0.001$ *versus* 0 wk; **, $P < 0.05$ *versus* 0, 2, and 4 wk. Comparison among groups: #, $P < 0.001$ *versus* diabetic + anti-VEGF Ab and control + anti-VEGF Ab; §, $P < 0.05$ *versus* diabetic + anti-VEGF Ab.

ies, vascular bundles in the outer medulla). In the glomeruli, the number of stained capillary profiles per glomerulus, normalized for glomerular surface area, was significantly higher in diabetic rats compared with control rats (12.6 ± 0.8 *versus* 9.1 ± 0.1; $P < 0.01$). The increased staining for eNOS in the glomerular capillary endothelial cells was also present in diabetic rats that were treated with control Ab (13.1 ± 1.33; $P < 0.05$ *versus* control) but not in anti-VEGF Ab-treated diabetic rats (8.1 ± 0.4; $P < 0.01$ *versus* diabetes and diabetes + control Ab; Figure 5).

Immunocytochemical Localization of the Anti-VEGF and Control Ab

No staining was detected in either glomeruli, tubules, or peritubular spaces, indicating that the anti-VEGF or control Ab did not accumulate in the kidney (data not shown).

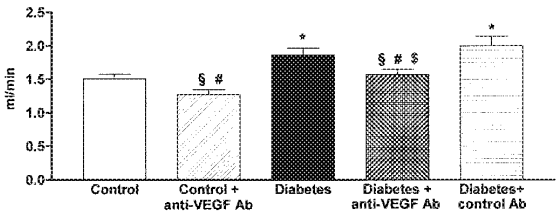


Figure 3. FITC-inulin clearance in untreated ($n = 12$) and anti-VEGF Ab-treated ($n = 7$) control rats and in untreated ($n = 11$), anti-VEGF Ab-treated ($n = 12$), and control Ab-treated ($n = 9$) diabetic rats. *, $P < 0.01$ *versus* control; §, $P < 0.05$ *versus* diabetes; #, $P < 0.01$ *versus* diabetes + control Ab; $, $P < 0.05$ *versus* control + anti-VEGF Ab.

Discussion

A potential role for VEGF in the pathophysiology of early diabetic nephropathy can be reasoned based on several lines of evidence. First, VEGF is constitutively expressed with a distinct glomerular localization, which suggests an important role in the regulation of glomerular permeability, blood flow, and endothelial cell growth (10). In the adult kidney, VEGF mRNA and protein expression is very pronounced in visceral epithelial cells (7–9). VEGF receptor mRNA and protein were detected in endothelial cells of glomerular capillaries (8,9,11) and pre- and postglomerular vessels (8). The finding that glomerular endothelial cells are a major binding site for exogenous ^{125}I-VEGF (9,11) is consistent with these observations. Although it remains unclear how VEGF crosses the glomerular basement membrane, it is reasonable to postulate the existence of a local regulatory mechanism, with generation of the peptide in podocytes, paracrine secretion, and binding on glomerular capillary endothelium.

Second, VEGF expression is increased in several cell types and tissues by high ambient glucose concentrations. High glucose levels in the culture medium upregulate VEGF expression in vascular smooth muscle cells (16), in retinal epithelial cells (17), and in glomerular endothelial cells (18). Upregulation of VEGF has been demonstrated in the retina of diabetic patients (19) and experimental animals (20). An increased renal expression of VEGF mRNA and protein was reported in

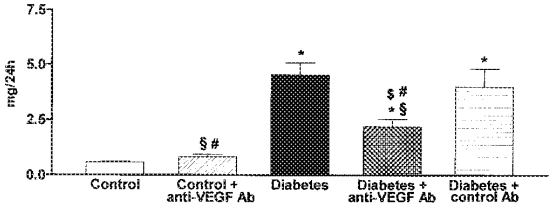


Figure 2. Albuminuria in untreated ($n = 12$) and anti-VEGF Ab-treated ($n = 7$) control rats and in untreated ($n = 11$), anti-VEGF Ab-treated ($n = 12$), and control Ab-treated ($n = 9$) diabetic rats. *, $P < 0.0001$ *versus* control; §, $P < 0.001$ *versus* diabetes; #, $P < 0.02$ *versus* diabetes + control Ab; $, $P < 0.01$ *versus* control + anti-VEGF Ab.

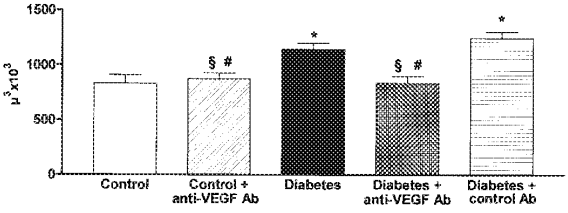
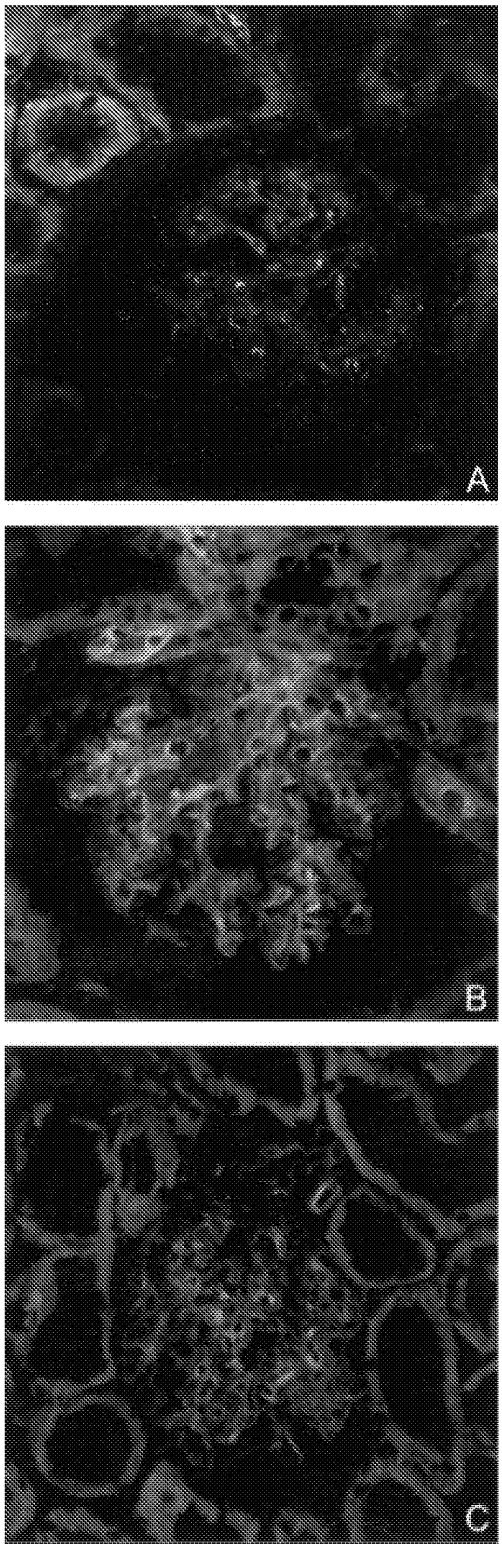


Figure 4. Glomerular volume in untreated ($n = 6$) and anti-VEGF Ab-treated ($n = 6$) control rats and in untreated ($n = 6$), anti-VEGF Ab-treated ($n = 6$), and control Ab-treated ($n = 6$) diabetic rats. *, $P < 0.01$ *versus* control; §, $P < 0.01$ *versus* diabetes; #, $P < 0.001$ *versus* diabetes + control Ab.

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experimental rat models of diabetes type I (11) and type II (12). In addition, the expression of VEGF receptors was found to be upregulated in kidneys of diabetic rats (11).

Finally, interference with the VEGF-NO axis was shown to prevent microvascular dysfunction induced by high glucose levels in the dorsal skinfold chamber of the rat (13).

Although these studies suggest a causal role for VEGF in the pathophysiology of diabetic nephropathy, the evidence remains circumstantial. The present data are the first to support an causative role for VEGF in the early renal changes in diabetes. Streptozotocin-induced diabetic rats and age-matched control rats were treated chronically with murine monoclonal anti-VEGF Ab or with isotype-matched control Ab, the latter to exclude an effect of immunization. Based on *in vitro* binding studies, an adequate serum VEGF inhibitory activity was achieved during the entire course of anti-VEGF Ab administration. In diabetic rats, anti-VEGF treatment partially prevented early renal dysfunction. The decrease in GFR in the anti-VEGF Ab-treated diabetic rats could not merely be attributed to precipitation of antigen-Ab complexes in the glomerular basement membrane, because no extravascular murine Ab was detected at the end of the experiment by immunohistochemical analysis. The effects were specific for diabetes, because the anti-VEGF Ab did not affect filtration rate and glomerular volume in control rats. These findings are consistent with a previous report that a $VEGF_{165}$ aptamer did not affect glomerular morphology in normal rats (21). The present results indicate that the upregulation of VEGF and its receptors in the diabetic kidney, as reported by others (11,12), contributes to the pathophysiology of early renal dysfunction in diabetes. The beneficial effect of VEGF-blockade does not exclude the involvement of other growth factors in the pathogenesis of early diabetic renal dysfunction. Inhibition of TGF-β (22,23) and interference with the growth hormone/IGF-I system (24,25) were shown recently to prevent manifestations of early experimental diabetic nephropathy. Several diabetes-induced mediators interact in their adverse effects on the kidney; therefore, it is not surprising that correction of any of them results in amelioration of diabetic nephropathy. In addition, it should be noted that the deleterious effects of VEGF may be limited to early diabetes. When diabetic vascular disease is full-blown and results in tissue ischemia, VEGF may actually be essential for collateral vessel formation (26).

Several recent studies provided evidence that VEGF exerts its angiogenic, vascular permeability, and hemodynamic ef-

Figure 5. Immunocytochemical staining for endothelial nitric oxide synthase (eNOS) in control (A), control Ab-treated diabetic (B), and anti-VEGF Ab-treated (C) kidneys. Staining is detectable in glomerular capillaries and in afferent and efferent arterioles, visible at the vascular pole of the glomeruli. As compared with control rats, the glomeruli of control Ab-treated diabetic rats are characterized by increased number of eNOS-positive capillary profiles. Treatment of diabetic rats with neutralizing anti-VEGF Ab results in a lower number of stained capillaries, which is not different from controls. Magnification, ×330.

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fects via upregulation of eNOS in endothelial cells (4–6). The action of NO as a downstream mediator of VEGF is commensurate with the upregulation of the NO system in early diabetes and its implication in the pathogenesis of early renal dysfunction. NOS blockade substantially reduces or even completely eliminates the hyperfiltration in experimental diabetes (27–29). Chronic administration of NG-nitro-L-arginine methyl ester to diabetic rats partially prevented the increase in glomerular volume in diabetic rats (30). In the present study, an increased expression of eNOS was documented in glomerular capillary endothelial cells of the diabetic rats, confirming earlier findings (27,30). VEGF blockade prevented the upregulation of eNOS in the diabetic glomeruli, thus supporting the contention that VEGF binds to its receptors on glomerular capillary endothelial cells and increases eNOS expression in these cells.

The mechanism(s) by which VEGF may affect glomerular permeability and filtration rate remains speculative. VEGF is known to induce fenestrations in endothelial cells *in vitro* (31), and it has been hypothesized that VEGF is involved in the induction and maintenance of the fenestrae in the glomerular capillary endothelial cells (9). It is, however, generally acknowledged that the capillary fenestrations do not represent the ultimate barrier for filtration but rather that the glomerular basement membrane and the podocyte foot processes with their interconnecting slit diaphragms restrict the passage of proteins. Alternatively, VEGF could affect the filtration barrier by increasing the production of NO in glomerular endothelial cells, which in turn diffuses to the podocytes and acts on the slit pores. In support of this hypothesis, local NOS blockade is known to reduce glomerular ultrafiltration coefficient (32). In addition, VEGF may increase glomerular filtration surface area by stimulating glomerular capillary endothelial cell growth. Nyengaard and Rasch (33) demonstrated that the increased glomerular filtration surface in diabetes results from a formation of new glomerular capillaries, in addition to a slight elongation of existing capillaries. This phenomenon is analogous to the capillary proliferation observed in diabetic retinas and in other vascular beds and thus may be understood as one expression of a generalized diabetic microangiopathy. Our finding that blockade of VEGF prevented the increase in glomerular tuft volume in diabetic rats supports the contention that VEGF is an essential growth factor for glomerular capillaries in pathophysiologic circumstances. In accordance, administration of a $VEGF_{165}$ aptamer was found to decrease glomerular endothelial cell regeneration in experimental glomerulonephritis (21).

The cause of the upregulation of VEGF in diabetes remains speculative, but multiple factors may be implicated. Several factors relevant to the pathogenesis of diabetic complications have been shown to promote VEGF expression in various cell types and tissues, including advanced glycation end products (34), angiotensin II (35), reductive stress (13), reactive oxygen species (36), TGF-β (37), and IGF-I (38). Importantly, protein kinase C (PKC), which is increasingly recognized as a central mediator of the damaging effects of hyperglycemia, has been shown to upregulate VEGF (16,39). Direct (40) or indirect (41) inhibition of PKC activation in diabetic rats produced similar improvement of glomerular hyperfiltration and albuminuria as in our study, further supporting a link between PKC and VEGF. Taken together, many factors could act independently or in combination to increase VEGF expression in the diabetic kidney. However, the *in vivo* relevance of these pathways remains to be determined. In addition to increased renal expression of VEGF and its receptors (11,12), a disturbed feedback regulation of VEGF could contribute to the pathophysiologic effects of VEGF in the diabetic kidney. Because VEGF is a molecule with very high potency, the existence of powerful defense mechanisms may be assumed. One such mechanism may be the binding of VEGF to the heparin sulfate glycosaminoglycan side chains in the glomerular basement membrane (42). It is tempting to speculate that the reduced heparin content of the glomerular basement membrane in diabetes (43) may contribute to an increased access of VEGF to its receptors.

In conclusion, blockade of VEGF has renoprotective effects in early streptozotocin-induced diabetes in rats. In comparison with untreated diabetic rats, animals that were treated with monoclonal Ab against VEGF exhibited a smaller increase in GFR, glomerular volume, and UAER. The present study demonstrates a new mechanism by which hyperglycemia causes renal dysfunction. Targeting VEGF may prove useful as a therapeutic strategy for the treatment of early diabetic nephropathy.

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Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases

See the related Commentary beginning on page 600.

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Kidney disease affects over 20 million people in the United States alone. Although the causes of renal failure are diverse, the glomerular filtration barrier is often the target of injury. Dysregulation of VEGF expression within the glomerulus has been demonstrated in a wide range of primary and acquired renal diseases, although the significance of these changes is unknown. In the glomerulus, VEGF-A is highly expressed in podocytes that make up a major portion of the barrier between the blood and urinary spaces. In this paper, we show that glomerular-selective deletion or overexpression of VEGF-A leads to glomerular disease in mice. Podocyte-specific heterozygosity for VEGF-A resulted in renal disease by 2.5 weeks of age, characterized by proteinuria and endotheliosis, the renal lesion seen in preeclampsia. Homozygous deletion of VEGF-A in glomeruli resulted in perinatal lethality. Mutant kidneys failed to develop a filtration barrier due to defects in endothelial cell migration, differentiation, and survival. In contrast, podocyte-specific overexpression of the VEGF-164 isoform led to a striking collapsing glomerulopathy, the lesion seen in HIV-associated nephropathy. Our data demonstrate that tight regulation of VEGF-A signaling is critical for establishment and maintenance of the glomerular filtration barrier and strongly supports a pivotal role for VEGF-A in renal disease.

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Introduction

Glomeruli are highly specialized filtration barriers between the blood and urinary space. Each day, approximately 180 l of blood passes through these filters in the average adult human kidney. Although water and small solutes must pass freely through this barrier, critical blood proteins such as albumin and blood clotting factors must not. The filter has a number of unique characteristics that provide the essential properties for this renal filtration process and include highly specialized glomerular visceral epithelial cells (podocytes), a fenestrated glomerular capillary endothelial system, and intervening glomerular basement membrane (GBM) that is produced by both the podocytes and the endothelial cells (1, 2) (Figure 1a). This filtration barrier is the target of injury and ultimate scarring in a wide variety of kidney diseases (3–8).

During glomerular development, the podocytes express numerous vascular growth factors such as VEGF-A, while the glomerular endothelial cells express the VEGF receptors fetal liver kinase 1 (Flk1) and fms-like tyrosine kinase 1 (Flt1) (9). In addition, the podocytes are geographically situated at the developing vascular cleft adjacent to incoming endothelial cells (2, 10) (Figure 1b). The location and gene expression profile of podocytes suggests that they are required to provide migratory cues to glomerular endothelial cells to establish the renal filtration barrier. Furthermore, similar to other fenestrated vascular beds in the body, podocytes continue to express VEGF-A in the mature glomerulus. This suggests that VEGF plays a role in maintaining the filtration barrier either through survival, proliferation, and/or differentiation cues to the adjacent specialized endothelia.

It is clear that VEGF is a critical mediator of vasculogenesis as heterozygous and homozygous null VEGF-A mice die with major vascular defects at 11.5 and 9.5 days after coitus, respectively (11, 12). However, its later role in specific vascular beds, such as the glomerulus, is less clear. Although dysregulation of VEGF-A expression

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Nonstandard abbreviations used: glomerular basement membrane (GBM); fetal liver kinase 1 (Flk1); fms-like tyrosine kinase 1 (Flt1); HIV-associated nephropathy (HIVAN); Wilms tumor suppressor gene (WT1); α-smooth muscle actin (VSMA).

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has been demonstrated in a number of renal diseases, the significance of these changes is presently unknown.

To determine the role of VEGF-A in the glomerular filtration barrier, we generated mice with gain or loss of function of VEGF specifically in the podocyte, thus avoiding the embryo-lethal effects. The distinct glomerular-specific haploinsufficient and null phenotypes observed in this study demonstrate for the first time that the "dose" of VEGF is critical in the establishment and maintenance of later vascular beds, as it is in vascular formation during earlier stages of embryogenesis.

By 2.5 weeks of age, mice with podocyte-specific heterozygosity for VEGF developed endotheliosis and "bloodless glomeruli," the renal lesion seen in preeclampsia, which progressed to nephrotic syndrome, a common glomerular syndrome seen in humans (13), and end-stage kidney failure by 9–12 weeks. The podocyte-specific homozygotes died at birth or within 18 hours of birth with hydrops (generalized swelling), kidney failure, and grossly abnormal glomeruli that lack mature endothelial cells. In contrast, overexpression of the 164 isoform of VEGF-A in podocytes also led to end-stage renal failure due to a collapsing glomerulopathy, which is the pathologic lesion seen in HIV-associated nephropathy (HIVAN) (14). This demonstrates that there is an ongoing requirement for tight regulation of VEGF signaling between the podocyte and glomerular endothelium; disruption in this regulation leads to dramatic and distinct renal phenotypes that are determined by the glomerular VEGF dose and suggests that VEGF is pivotal in a wide variety of renal diseases.

Methods

Cell-specific gene targeting. Three independent podocyte-specific Cre recombinase murine lines, A15, GG8, and V9, which all demonstrated 100% excision when crossed to the Z/EG reporter mouse strain (15), were bred to the floxed VEGF-A mouse (Figure 1c). The VEGF-A mouse has loxP sites inserted around the third exon (16). Site-specific recombination between the loxP sites of the VEGF gene results in a null VEGF allele (16).

To generate homozygous floxed VEGF–Cre recombinase mice, bitransgenic mice carrying both a nephrin-Cre transgene and one floxed VEGF-A allele were bred to homozygous floxed VEGF-A mice.

To generate transgenic founders that overexpress the 164 isoform of VEGF-A, a 645-bp fragment of the VEGF gene, including a Kozak consensus sequence (nucleotides 78–669 of GenBank accession no. NM009505) and initiating ATG, were subcloned into the XhoI and XbaI sites of a pNXRS vector between a 4.125-kb 5′ fragment of the murine nephrin gene that is capable of podocyte-specific expression in vivo in the kidney (17) and a 0.97-kb poly(A) signal from the SV40 polyoma virus (Figure 1e).

Genotyping. Genomic DNA was isolated from mouse tails as described. The nephrin-Cre transgenic mice were generated and genotyped as previously described (17). Floxed VEGF mice were received from Napoleone Ferrara (Genentech Inc.). Presence of the floxed VEGF gene was detected by PCR using the oligonucleotide primers muVEGF 419.F (5′-CCTGGCCCTCAAGTACACCTT-3′) and muVEGF 567.R (5′-TCCGTACGACGCATTTCTAG-3′) (both from Sigma-Genosys, The Woodlands, Texas, USA), which generate a 148-bp fragment of the VEGF allele in the presence of the loxP-1 site and a DNA fragment that is approximately 40 bp shorter than for the wild-type allele (16).

To identify founder mouse lines that carried the nephrin-VEGF-164 transgene, Southern blot analysis was performed. Briefly, the DNA was digested with EcoRI; the probe used was the 645-bp fragment encoding the VEGF-164 cDNA that recognized a 1.3-kb genomic fragment in the transgenic founders. To estimate transgene copy number, 1 μg, 2 μg, and 5 μg of genomic DNA from the transgenic founder or wild-type mice was blotted on Biodyne B membrane (P/N 60207, Pall Gelman Laboratory, Ann Arbor, Michigan, USA) and hybridized with the VEGF cDNA probe described above. The signal was quantified using the Quantity One quantitation software program (4.2.1 version) (Bio-Rad Laboratories, Hercules, California, USA) according to the manufacturer's instructions.

Phenotypic analysis. Urine was collected passively in an Eppendorf tube from 0-, 3-, 6-, and 9-week-old mice. A urine dipstick (Chemstrip 5L; Roche Diagnostics Corp., Indianapolis, Indiana, USA) was used to detect the presence or absence of protein and red blood cells in the urine. The standard colorimetric assay was performed according to the manufacturer's instructions. In addition, 2 μl of urine from transgenic or control mice was placed in 18 μl of Laemmli buffer (18), boiled, and loaded on a 12% SDS-PAGE gel. An SDS-PAGE low-range protein standard (Bio-Rad Laboratories Inc., Hercules, California, USA) was loaded in the first lane of the gel.

Blood samples were taken with a heparinized capillary tube by femoral vein stab after warming. A total of 120 μl of blood was collected and creatinine, urea, and blood chemistry measurements were recorded using a Stat Profile M7 (Nova Biomedical Corp., Waltham, Massachusetts, USA). The CBC (total blood count) was performed on a Coulter Counter (AcT diff; Beckman Coulter Canada, Ontario, Canada).

Histologic analysis. Embryonic tissues for histologic analysis were dissected, fixed in 10% formalin/PBS, and embedded in paraffin. Sections 4 μm thick were cut. Sections were stained with H&E, examined, and photographed with a DC200 Leica camera and Leica DMLB microscope (Leica Microsystems Inc., Deerfield, Illinois, USA). Tissue for electron microscopy was fixed in 1.5% glutaraldehyde, embedded in Spurr (Canemco Inc., Saint-Laurent, Quebec, Canada), and sectioned.

In situ hybridization and immunohistochemistry. Kidneys were dissected from mice on postnatal day 0 and at 1 week, 3 weeks, 6 weeks, or 9 weeks of age. Kidneys were

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washed briefly in RNase-free PBS and fixed overnight in DEPC-treated 4% paraformaldehyde. These tissues were then placed in 30% sucrose for 12–24 hours, embedded in Tissue-Tek OCT 4583 compound (Sakura Finetek USA Inc., Torrance, California, USA) and snap frozen. Ten-micron tissue samples were cut on a Leica Jung cryostat (model CM3050; Leica Microsystems Inc.) and transferred to Superfrost microscope slides (Fisher Scientific Co., Pittsburgh, Pennsylvania, USA). The slides were stored at –20°C until needed. Digoxigenin-labeled probes were prepared according to the Roche Molecular Biochemicals protocol (Roche Molecular Biochemicals, Mannheim, Germany). Probes used for in situ analysis were nephrin (19), Wilms tumor suppressor gene (WT1; a kind gift of J. Kreidberg, Children's Hospital, Boston, Massachusetts, USA), podocin (a kind gift from C. Antignac, Institut National de la Santé et de la Recherche Médicale, Paris, France), α-smooth muscle actin (VSMA; a kind gift from P. Igarashi, Southwestern University, Dallas, Texas, USA), VEGF-A (kind gift of A. Nagy, Samuel Lunenfeld Research Institute, Toronto, Canada). Details of the in situ analysis protocol may be obtained upon request.

Immunostaining was performed with antibodies to WT1 and PECAM as described (20).

Results

Mice that are heterozygous for VEGF-A in the podocyte develop endotheliosis and nephrotic syndrome. To determine whether there is any phenotype resulting from a reduction in VEGF-A gene dose within the podocyte, we used the Cre-loxP system. Nephrin-Cre recombinase mice were generated in our laboratory and are capable of site-specific recombination in 100% of podocytes at the capillary-loop stage in vivo (17). We generated mice that were heterozygous for the floxed VEGF-A allele and carried the nephrin-Cre transgene *VEGF-loxP*$^{+/-}$*,Neph-Cre*$^{+/-}$

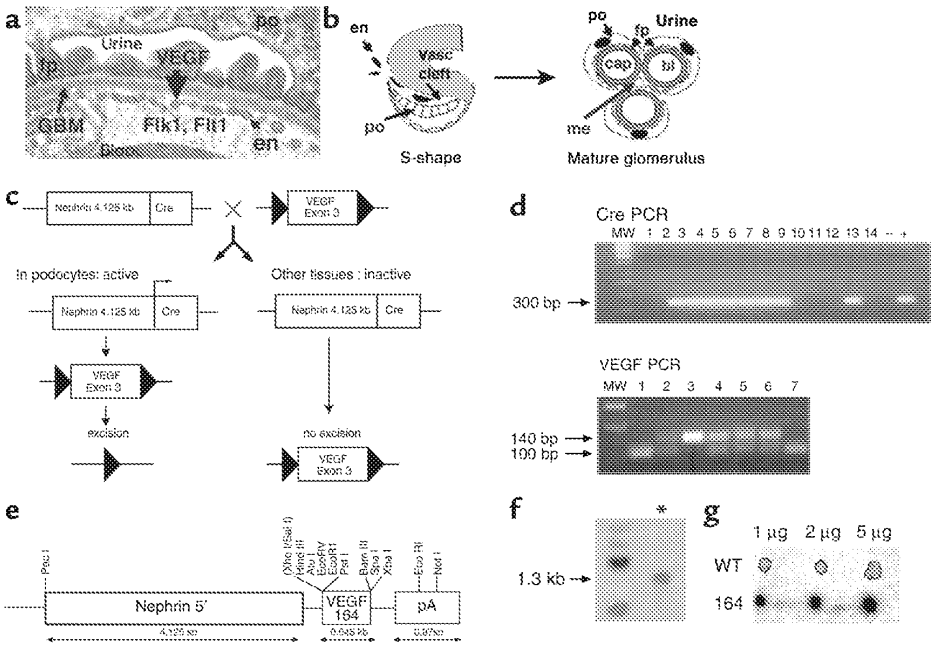


Figure 1

Expression and genomic targeting of VEGF-A within the glomerular filtration barrier. (**a**) Transmission electron micrograph of the glomerular filtration barrier that consists of podocytes (po) and their specialized foot processes (fp), fenestrated endothelium (en), and intervening GBM. VEGF-A is produced in the podocyte; the VEGF receptors Flk1 and Flt1 are expressed in the adjacent endothelial cells. (**b**) Development of the glomerular filtration barrier. In the S-shape stage, podocyte precursors (po) express VEGF-A. Endothelial cells (en) that express the VEGF receptors migrate into the vascular (Vasc) cleft and differentiate in direct apposition to podocytes. In the mature glomerulus, the fenestrated endothelial capillary loops (cap) remain in intimate contact with the VEGF-expressing podocytes (po). Mesangial cells (me) provide support to the capillary tuft. Urine is formed as blood (bl) is filtered from the capillaries, across the GBM, and through slit diaphragms that connect adjacent podocyte foot processes (fp). (**c**) Scheme to generate heterozygous and homozygous podocyte-specific VEGF knockout mice. Triangles are 34 bp loxP sites. (**d**) The Cre recombinase transgene was identified as a 300 bp PCR product. The floxed VEGF allele measures 140 bp by PCR analysis, whereas the wild-type allele measures 100 bp. MW, molecular weight markers. (**e**) Transgenic construct used to overexpress the 164-isoform of VEGF. pA, poly(A). (**f**) Presence of the transgenic VEGF-164 gene was identified as a 1.3-kb band (*) by Southern blot analysis. (**g**) Dot blot analysis of transgene copy number. The transgenic founder mice (164) demonstrated a 30-fold increase in copy number compared with the wild type.

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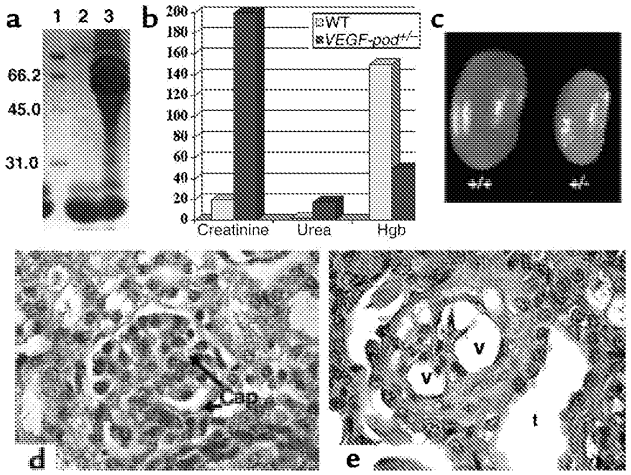


Figure 2

Heterozygous *VEGF-loxP*$^{+/-}$,*Neph-Cre*$^{+/-}$ mice develop nephrotic syndrome and end-stage renal failure by 9 weeks of age. (**a**) SDS-PAGE analysis was performed using 2 μl of mouse urine. Lane 1 contains molecular weight markers, lane 2 shows urine from a *VEGF-loxP*$^{+/-}$,*Neph-Cre*$^{+/-}$ control aged 9 weeks, and lane 3 shows urine from a 9-week-old sick *VEGF-loxP*$^{+/-}$,*Neph-Cre*$^{+/-}$ animal. The presence of a large amount of albumin measuring 66.2 kDa is identified in the sick mouse and demonstrates damage to the kidney filter. In contrast, low–molecular-weight proteins, which are normally found in mouse urine, are not different. (**b**) Bar graph showing elevated creatinine (more than ten times higher than normal), elevated urea, and decreased hemoglobin (Hgb) in *VEGF-loxP*$^{+/-}$,*Neph-Cre*$^{+/-}$ mice (VEGF-*pod*$^{+/-}$) at 9 weeks of age compared with *VEGF-loxP*$^{+/-}$,*Neph-Cre*$^{-/-}$ and *VEGF-loxP*$^{-/-}$,*Neph-Cre*$^{+/-}$ mice (combined for analysis and considered as wild type). (**c**) Whole-mount image of a kidney from a sick 9-week-old *VEGF-loxP*$^{+/-}$,*Neph-Cre*$^{+/-}$ (+/−) mouse compared with that of a wild-type littermate (+/+). The affected kidney is pale and shrunken. Magnification: ×60. (**d**) A wild-type glomerulus. Note the open capillary loops (Cap). ×350. (**e**) A glomerulus from a heterozygous VEGF-A mouse. All the glomeruli are grossly distorted morphologically. Note the empty cytoplasmic vacuoles (v) that are present in podocytes. No patent capillary loops can be seen. Dilated tubules (t) can be seen and in most places are packed with proteinaceous material, consistent with nephrotic syndrome. Magnification: ×375.

(Figure 1, c and d). They were born in the expected mendelian frequency but developed end-stage kidney failure by 9–12 weeks of age.

Physical examination of mice at this stage showed that 30/30 of the *VEGF-loxP*$^{+/-}$,*Neph-Cre*$^{+/-}$ mice had lethargy and decreased skin turgor. Urinalysis was performed and showed 3.0 g/l of protein (defined as "nephrotic range" proteinuria) and 250 red blood cells/μl of urine by dipstick analysis. SDS-PAGE analysis demonstrated massive albuminuria in all of these *VEGF-loxP*$^{+/-}$,*Neph-Cre*$^{+/-}$ mice (Figure 2a), which is pathognomonic for damage to the filtration barrier. Blood chemistry showed mice to have severely decreased renal function with an elevated serum creatinine that measured 200 μM, more than ten times the normal value, markedly elevated urea, and a normochromic, normocytic anemia consistent with end-stage kidney failure (Figure 2b). Of note, fragmentation of red blood cells was not observed on the blood smear. The mice did not demonstrate any gross signs of renal failure prior to 7–8 weeks of age.

At 9 weeks of age, the kidneys were pale and shrunken (Figure 2c). By light microscopy, the glomeruli looked histologically normal at birth and at 1, 3, and 6 weeks of age. However, by 9 weeks of age, the glomerular tufts were retracted with expansion of the mesangial matrix and were surrounded by podocytes containing large empty cytoplasmic vacuoles. The tubules were packed with protein (Figure 2e and data not shown).

Serial transmission EM studies demonstrated that the first detectable lesion occurred at 2.5 weeks of age with swelling of the endothelial cells (endotheliosis) and hyaline deposits (Figure 3a) that resemble the pathologic lesions seen in renal biopsies from patients with preeclampsia, a common disease of pregnancy (21). At this time, the podocytes and endothelial cells appeared ultrastructurally normal with well-formed foot processes and fenestrations, respectively. By 6.5 weeks of age, the GBM was expanded and endothelial fenestrations could no longer be identified (Figure 3b). By 9 weeks of age, the endothelial cells were necrotic and no podocyte foot processes could be identified (Figure 3b).

Molecular marker analysis confirmed the disappearance of differentiated podocytes with a complete absence of WT1, nephrin, and VEGF-A in the majority of glomeruli of terminally ill mice (Figure 4, g and h, and data not shown). On occasion, a single cell could be identified that stained positively for these markers (Figure 4h). These markers were all present at birth and at 3 and 6 weeks of age (Figure 4, d–f and data not shown). The level of VEGF-A mRNA was consistently lower in the heterozygous VEGF glomeruli than in control glomeruli at the same developmental stage (Figure 4d). VSMA is not normally found in 9-week-old mesangial cells unless they are "activated" in glomerular injury; in the heterozygotes, occasional VSMA-positive cells were identified in the glomeruli (Figure 4i).

VEGF-A is required in the podocyte to establish the glomerular filtration barrier. In order to investigate the phenotype resulting from a complete absence of VEGF-A in the glomerulus, mice that were null for VEGF-A specifically in the podocyte (*VEGF-loxP*$^{+/+}$,*Neph-Cre*$^{+/-}$ mice) were generated (n = 15). These mice were born at the expected mendelian frequency but died at birth or within 18 hours of birth. Some of these mice were born with hydrops that can be seen in infants with congenital nephrotic syndrome (22).

Light microscopy demonstrated that all of the null VEGF-A glomeruli were small with no or few distin-

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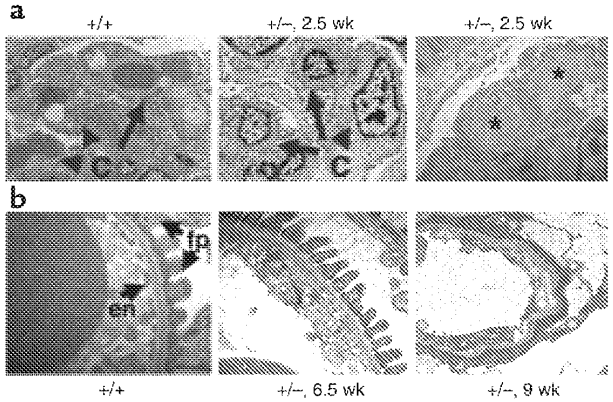


Figure 3
Heterozygous *VEGF-loxP*$^{+/-}$,*Neph-Cre*$^{+/-}$ mice demonstrate endotheliosis and loss of fenestrations. (**a**) At 2.5 weeks of age, wild-type glomerular capillary loops (c) are open and contain numerous red blood cells. In contrast, podocyte-specific VEGF-A heterozygotes (+/–) demonstrate bloodless glomeruli, and the capillary loops are filled with swollen endothelial cells, demonstrating endotheliosis, the classic renal lesion of preeclampsia. In addition, large subendothelial hyaline deposits (*) can be seen. (**b**) At 6.5 weeks of age, wild-type filtration barriers (+/+) are characterized by fenestrated endothelial cells (en) and well-formed podocyte foot processes (fp). In the podocyte-specific heterozygotes (+/–), the fenestrations are lost at 6.5 weeks of age, and by 9 weeks of age, the endothelial cells appear necrotic and no podocyte foot processes can be identified.

guishable glomerular capillary loops. Additionally, podocytes were present but tended to pile up in several layers and lacked well-formed slit diaphragms, the specialized intercellular junctions found between foot processes (Figure 5a and data not shown).

Immunohistochemical analysis with an antibody to PECAM that recognizes a cell surface receptor on endothelial cells was performed. Although endothelial cells were present in most immature glomeruli, they were markedly reduced in number and mature glomeruli lacked endothelial cells altogether (Figure 5b). BrdU labeling was performed; labeled endothelial cells were easily identified in the vascular clefts of wild-type S-shape stage glomeruli but were never observed in podocyte-specific VEGF-A null glomeruli (data not shown).

EM studies demonstrated widespread but not complete effacement of podocyte foot processes (not shown). Small capillary loops with a GBM could be identified in some glomeruli. However, this GBM failed

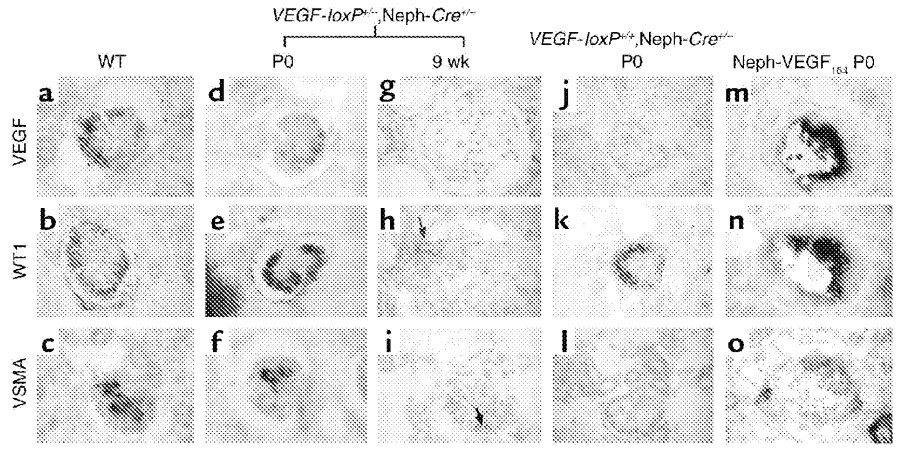


Figure 4
Digoxigenin-labeled in situ analysis of wild-type and mutant glomeruli. (**a**–**c**) Capillary loop–stage glomeruli from a newborn *VEGF-loxP*$^{+/-}$, *Neph-Cre*$^{+/-}$ control mouse demonstrate expression of VEGF-A and WT1 in podocytes, while VSMA is expressed in mesangial cells, which are found inside the glomerulus and are required to support the capillary structure. (**d**–**f**) At birth (P0), capillary loop–stage glomeruli from a heterozygous *VEGF-loxP*$^{+/-}$,*Neph-Cre*$^{+/-}$ mouse demonstrate normal levels of expression of WT1 and VSMA, while VEGF expression is consistently reduced at the mRNA level compared with the wild-type controls. (**g**–**i**) By 9 weeks of age, the heterozygous VEGF mice are clinically unwell. At this time, most glomeruli demonstrate a complete absence of markers of podocyte differentiation (i.e., no VEGF or WT1; both are absent). In **h**, a single WT1-positive cell can be identified (arrow). (**i**) VSMA is not usually present in glomeruli at 9 weeks; however, occasional VSMA-positive cells can also be identified and likely represent "activated" mesangial cells (arrow). (**j**–**l**) In the null *VEGF-loxP*$^{+/+}$,*Neph-Cre*$^{+/-}$ glomeruli at birth, no VEGF is seen in glomeruli as predicted due to podocyte-specific excision of both VEGF alleles. WT1 is present in differentiated podocytes. In contrast, VSMA is absent, demonstrating a defect in migration and/or differentiation of mesangial cells into the glomerulus. (**m**–**o**) In the nephrin–VEGF-164 mouse, both VEGF and WT1 are expressed in podocytes present within collapsed glomeruli. VEGF is markedly upregulated. In addition, VSMA and mesangial cells are present but appear to surround the collapsed glomerulus in a crescent shape. Magnification: ×350.

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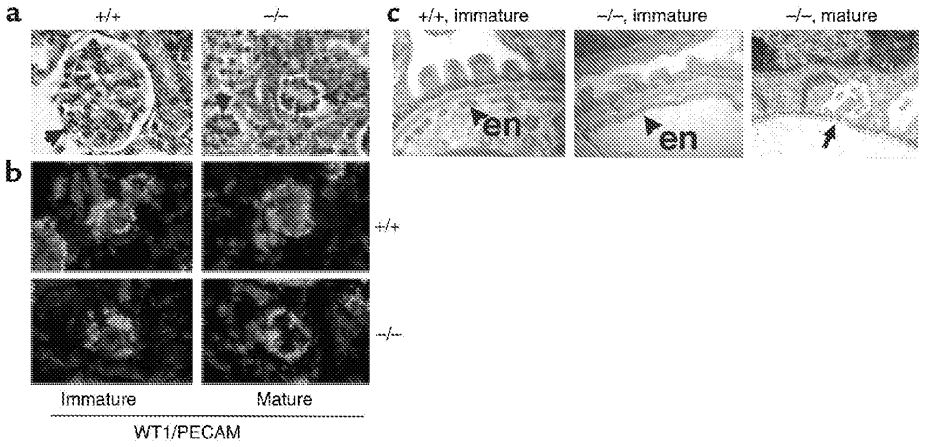


Figure 5
VEGF-null glomeruli do not form filtration barriers or fenestrations within endothelial cells. (**a**) The wild-type (+/+) glomerulus (arrow) has a lacy appearance due to open capillary loops. The VEGF-null glomeruli (−/−) fail to develop fully and lack visible capillary loops. Magnification: ×350. (**b**) Immunohistochemical staining for WT1 (green), a marker for podocyte cells, and PECAM (red), a marker for endothelial cells, shows a reduced number of endothelial cells in immature (capillary loop–stage) VEGF-null glomeruli. In mature glomeruli, no endothelial cells remain. Magnification: ×300. (**c**) Transmission EM of the filtration barrier in a wild-type (+/+) glomerulus clearly demonstrates fenestrated endothelium at the late capillary-loop stage, whereas no fenestrations are observed in endothelial cells (en) found in corresponding late capillary loop–stage VEGF-null glomeruli. In mature VEGF-null glomeruli, the basement membrane is seen (arrow), but the endothelial cells are missing. Magnification: ×20,000.

to fuse (data not shown). Endothelial cells were seen only rarely in capillary loop glomeruli and always lacked fenestrations (Figure 5c). In contrast, fenestrations were easily observed in endothelial cells of capillary loop–stage wild-type glomeruli (Figure 5c). Capillary loops in fully differentiated (mature) glomeruli demonstrated an absence of endothelial cells (Figure 5c).

In situ analysis showed that the podocytes that were present expressed markers of differentiation appropriately, including WT1 (Figure 4k), nephrin, and podocin (data not shown), although VEGF-A was absent due to genomic excision of the VEGF gene (Figure 4j). Of note, VSMA, a marker for mesangial cells, was absent from mutant glomeruli, although some desmin staining could be identified (Figure 4l and data not shown).

Upregulation of VEGF-A in podocytes leads to a collapsing glomerulopathy and death at 5 days of age. Given the distinct phenotypes observed when the dosage of VEGF is reduced by excising one or both alleles from the podocyte, we next sought to determine the effect of increasing the level of VEGF within the podocyte and its effect on the adjacent endothelium. Transgenic founder lines that overexpressed the 164 isoform of VEGF-A (nephrin–VEGF-164) under regulation of a 4.125-kb podocyte-specific promoter from the murine nephrin gene (Figure 1e) were identified by Southern blot analysis (Figure 1f). Two independent founder mice were used for analysis. By dot blot analysis, each of these founder mice demonstrated a 30-fold increase in the VEGF copy number (Figure 1g).

The transgenic mice appeared normal at birth but became growth-retarded within 2 days. By 5 days of age, the mice were clinically unwell and demonstrated albuminuria by dipstick analysis.

Grossly, the kidneys appeared normal in size, were hyperemic, and demonstrated cortical hemorrhages (Figure 6, a and b). Light microscopy showed global collapse of the glomerular tuft and dilation of proximal tubules that were packed with protein (Figure 6d and data not shown). Complete collapse of the capillary loops was illustrated by silver methenamine staining that recognizes the GBM (Figure 6f).

The few visible patent capillary loops were larger in diameter (Figure 6h) and multiple endothelial cell nuclei were visible within them (Figure 6, h and j) that were not seen in wild-type glomeruli (Figure 6, g and i). Although multiple endothelial cell nuclei could be identified within the few remaining patent glomerular capillary loops by EM, virtually all of the loops were collapsed and no endothelial cells could be identified. In addition, podocytes were abnormal and could be seen detaching from the GBM (data not shown).

In situ analysis confirmed that the majority of cells within the collapsed tufts were podocytes that continued to express WT1 (Figure 4n) and nephrin (not shown) and very high levels of VEGF-A that were upregulated five- to tenfold (Figure 4m and data not shown). Although mesangial cells were present as indicated by the presence of VSMA (Figure 4o), they were situated in a crescent shape at the periphery of the glomerulus.

Discussion

VEGF-A is a critical mediator of angiogenesis and vasculogenesis (11); both heterozygous and homozygous

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knockout mice die during embryogenesis due to major vascular defects. This demonstrates a dosage sensitivity for VEGF during development in the whole embryo (11). Other studies have shown that VEGF-A is required for the establishment and maintenance of endothelial fenestrae in vitro (23, 24).

Given the expression pattern of VEGF-A in developing and mature podocytes, which are located in direct apposition to fenestrated endothelial cells, and the fact that VEGF-A expression is associated with a variety of renal diseases, we hypothesized that VEGF-A is required in developing podocytes to establish and maintain the filtration barrier. To test this hypothesis, we used the Cre-loxP system to manipulate levels of VEGF-A expression specifically within the podocyte. The mice developed distinct haploinsufficient, null, and overexpression phenotypes. Thus, similar to vascular development in the early embryo, tight regulation of VEGF signaling is essential in the establishment of later vascular beds such as the glomerulus. In addition, sequential reduction in VEGF-A levels led to a loss of fenestrations or failure of fenestrations to form, definitively demonstrating for the first time a role for VEGF-A in maintenance of endothelial fenestrations in vivo. Finally, the distinct and dramatic renal phenotypes observed with each alteration of VEGF level suggest that VEGF signaling is pivotal in glomerular health and establish its role in the pathogenesis of glomerular disease.

Complete loss of VEGF-A in the glomerulus was 100% fatal in the perinatal period. The null *VEGF-loxP*$^{+/+}$,*Neph-Cre*$^{+/-}$ mice died within 18 hours of birth with generalized swelling (hydrops) and a failure of the glomerular filtration barrier to form. Although occasional endothelial cells were identified in most but not all capillary loop–stage (immature) glomeruli, all of these endothelial cells lacked fenestrations. Upon glomerular maturation, no endothelial cells remained. The variability in this phenotype is likely due to the time of genomic excision of the VEGF-A floxed allele. VEGF starts to be expressed during the S-shape stage of glomerulogenesis, whereas nephrin-Cre–mediated excision takes place slightly later during the capillary loop stage (17). Thus it appears that transient expression of VEGF is sufficient to direct a reduced number of incoming endothelial cells but insufficient to maintain survival and proliferation of these cells. In addition, our results suggest that there is a threshold level required for VEGF to establish fenestrations that is not reached in VEGF-null glomeruli. VSMA, a marker of glomerular mesangial cells during glomerular development, was also absent from null glomeruli, demonstrating that mesangial cell differentiation and/or

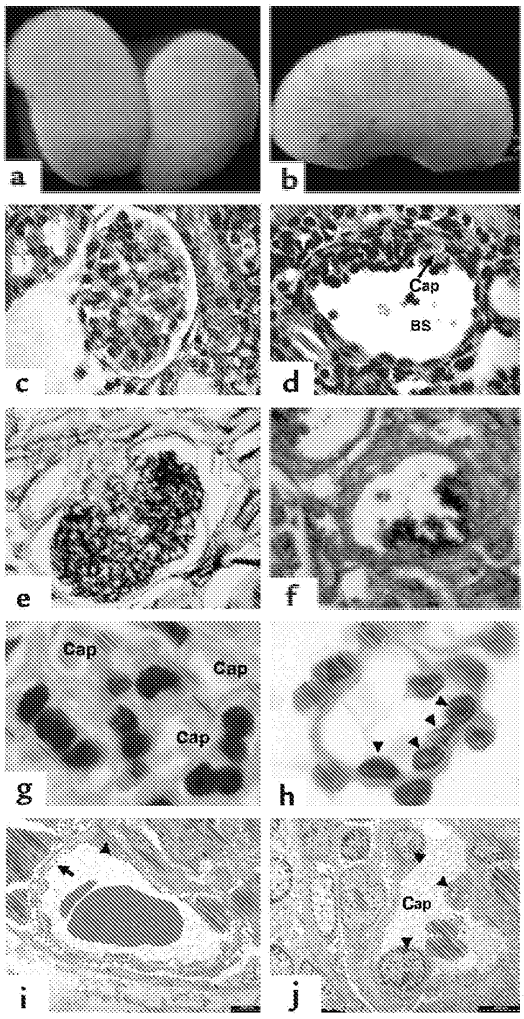


Figure 6
Mice that overexpress the 164 isoform of VEGF-A in their podocytes develop collapsing glomerulopathy. (**a** and **b**) Whole-mount images of VEGF-overexpressing kidneys at 5 days. The kidneys demonstrate many surface hemorrhages. (**c**) A glomerulus stained with H&E from a wild-type littermate. (**d**) A glomerulus from a transgenic VEGF-overexpressing mouse demonstrates global collapse of the capillary tuft toward the vascular pole of the glomerulus. A single patent capillary loop that appears dilated is identified (Cap). In addition, Bowman's space (BS) is enlarged. (**e**) A 5-day-old wild-type glomerulus is stained with silver methenamine that recognizes basement membranes (black). Note the intricate pattern of GBM that lines the capillary loops between endothelial cells and podocytes. (**f**) In contrast, a transgenic glomerulus demonstrates complete collapse of the capillary network. (**g**) A high-power view of the capillary loops (Cap) in a wild-type glomerulus. (**h**) In contrast, the few patent capillary loops identified at 5 days of age in the transgenic mice demonstrate increased diameter and multiple endothelial cell nuclei (arrowheads). (**i**) A wild-type capillary loop at 5 days of age. Note the fenestrated endothelium (arrow). Although a portion of an endothelial cell body is identified (arrowhead), glomerular endothelial cell nuclei are difficult to find on EM sections. (**j**) In a transgenic patent capillary loop at 5 days of age, three endothelial cell nuclei are easily identified (arrowheads). Magnification in **a** and **b**: ×60; in **c**–**f**: ×225; in **g** and **h**: ×1,000. In **i**, bar = 2,000 nm; in **j**, bar = 5,000 nm.

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migration is dependent upon successful establishment of a glomerular capillary system.

In contrast, initial development of the glomerular filtration barrier was unaffected in the heterozygous *VEGF-loxP*$^{+/-}$,*Neph-Cre*$^{+/-}$ mice. However, by 2.5 weeks of age, marked swelling of the glomerular endothelial cells led to the appearance of bloodless glomeruli and endotheliosis, the pathognomonic lesion seen in preeclampsia. Although preeclampsia is a common and potentially fatal disease that affects 7–8% of all pregnancies, the pathogenesis of this disorder is poorly understood. Patients typically develop proteinuria, and renal biopsies performed early in the disease demonstrate endotheliosis that progresses to glomerulosclerosis in a subset of patients (21). By 9 weeks of age, all of the podocyte-specific VEGF heterozygotes developed end-stage kidney failure due to a severe form of glomerulosclerosis with loss of differentiated podocytes and endothelial cells. Although alterations in circulating levels of VEGF have been implicated in preeclampsia (25), the significance of these changes is unknown and has not been studied in tissues of affected organs such as the kidney. Our results suggest that downregulation of VEGF signaling within the glomerulus may be involved in the renal lesion of preeclampsia. Because the primary defect in preeclampsia is believed to lie in the placenta and/or trophoblast, it is interesting to speculate that some as-yet-unidentified factor that is generated by the placenta leads to downregulation of VEGF-A expression within the glomerulus and endotheliosis, and suggests future areas of potential investigation.

Although glomerular defects were not observed prior to 2.5 weeks of age, it is quite possible that earlier endothelial and/or podocyte defects exist that we were unable to detect. Furthermore, the dramatic loss of podocytes by 9 weeks of age suggests that upon stimulation with VEGF-A, endothelial cells "signal back" to the podocyte, and that endothelial cell damage disrupts these reciprocal signals, emphasizing the importance and dependence of reciprocal interactions between these two cell types.

In addition to its paracrine role in the glomerulus, it is possible that VEGF-A has an autocrine function that is required for podocyte survival. Presently, it is controversial whether Flk1 is expressed even at low levels in podocytes. However, we have crossed our nephrin-Cre recombinase mice with floxed Flk1 mice (a kind gift of J. Rossant's lab at The Samuel Lunenfeld Research Institute). By 4 weeks of age, these mice have no phenotype that demonstrates an absence of Flk1–dependent autocrine signaling within the podocyte. VEGF-A is required for breast cancer cell survival in vitro; in this setting, VEGF-A appears to signal in an autocrine fashion through the VEGF coreceptor, neuropilin-1, in the absence of Flk1 (26). As neuropilin-1 is expressed in the podocyte, additional studies that target neuropilin-1 in the podocyte are required to definitively answer this question.

Previous studies that have globally reduced the expression of VEGF-A in the mouse by using neutralizing antibodies (16, 27) or expressing only the 120 isoform of VEGF-A (28) have reported glomerular defects that are different from those seen in our study. The intraperitoneal injection of neutralizing antibodies to human recombinant VEGF in postnatal day 1–3 mice led to mesangiolysis and an arrest in postnatal kidney development. Similarly, the postnatal administration of a soluble chimeric VEGF receptor (Flt1) led to hypocellular glomeruli with mesangial deposits and mesangial cell vacuolization (16). In addition, the authors observed a decrease in the number of glomerular capillaries and fewer endothelial cell fenestrations (16). In the developing kidney, VEGF-A is expressed both in podocytes and in tubular epithelial cells and adjacent metanephric mesenchymal cells (29). The differences seen between previous studies and the present one are most likely due to alteration of VEGF levels in multiple cell populations within the kidney and to a variable reduction of the VEGF dose, which may be more difficult to control with a circulating antibody or receptor. In addition, endogenous VEGF-A levels were upregulated in podocytes in a study by Kitamoto et al. (27). In our model, there is a complete absence of VEGF in podocytes. The glomerular phenotype was more severe in our null mice than in mice treated with blocking antibodies or the soluble Flt receptor, suggesting that the localized delivery of VEGF from the podocyte across the heparan sulfate–rich GBM to the VEGF receptors that face the GBM (30) is critical for its function in vivo.

More recently, Carmeliet and colleagues have reported that mice that express only the secreted 120 isoform of VEGF-A develop glomerulosclerosis by 6 weeks of age (28). Although endothelial cells are lost, the podocytes are reportedly normal. In our experiments, all isoforms of VEGF-A are lost from the kidney and the phenotype differs from the VEGF-120 mice. Together, these results clearly emphasize the importance of isoform-specific functions of VEGF-A within the glomerulus. Genomic targeting experiments that will address the role of the different VEGF isoforms within the podocyte are underway.

Given the exquisite sensitivity to VEGF dosage reduction in glomerular development and function, we also sought to determine the phenotype resulting from overexpression of the 164 isoform of VEGF-A specifically in the podocyte. The 164 isoform is secreted and bound by heparan sulfate in the GBM. These transgenic mice developed a dramatic glomerular phenotype and rapidly succumbed to end-stage renal failure. At the time of death, the majority of their glomeruli demonstrated global collapse of the tuft as seen in collapsing glomerulopathy and HIVAN (14). Why do the capillaries collapse? At birth and from 1–5 days of age, the glomeruli are present and filter urine. At this time, the patent glomerular capillary loops have greater diameters and a greater number of

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endothelial cell nuclei than do wild-type capillaries. Other studies have shown that treatment of endothelial cells with increasing doses of VEGF-A leads to coalescence of endothelial cells and the formation of larger endothelial tubes, a process that has been termed "hyperfusion" (31, 32). In the absence of increased glomerular capillary flow, this would lead to a fall in intraluminal capillary pressure and collapse. Of clinical relevance, the tat protein from HIV has been shown to signal through Flk1 in endothelial cells in Kaposi sarcoma (33–35), and the podocyte has been identified as a reservoir for the HIV virus (36). Taken together, these results present a possible explanation for the similarity between the capillary collapse seen in the VEGF-A overexpression model and HIVAN.

It has been hypothesized that damage to the podocyte ultimately leads to the capillary collapse seen in HIVAN and other forms of collapsing glomerulopathies (37–40). However, our results demonstrate that capillary collapse can occur in the absence of dedifferentiation or dysregulation of podocytes. In fact, the capillaries also collapse in heterozygous *VEGF-loxP*$^{+/-}$*;Neph-Cre*$^{+/-}$ mice after the endothelial cells are lost, and in this case, the differentiated podocytes are lost. Thus, it is evident that a single mechanism or phenotype cannot explain all cases of capillary collapse in glomerular disease.

In summary, our results demonstrate an exquisite dosage sensitivity for VEGF-A in the developing glomerulus. Numerous clinical studies have documented that alterations in glomerular VEGF-A expression are associated with glomerular disease (41–44). Our results demonstrate that dysregulation of VEGF-A is not only associated with but also plays a pathogenic role in initiating glomerular injury. The Cre-loxP system and transgenic approach allowed us to engineer mice with three different doses of VEGF within the podocyte based on the allele copy number. Each VEGF level was associated with a distinct mechanism that led to one of three important glomerular phenotypes. These results provide insight into the molecular mechanisms that underlie a variety of common and clinically important human diseases, including preeclampsia and HIV, and suggest potential future avenues for therapeutic intervention. In addition, it is clear that interactions between podocytes and endothelial cells are critical during development of the glomerular filtration barrier and continue in the filtering glomerulus.

Finally, these results provide a note of caution for clinical trials aimed at altering VEGF levels. Although the podocyte has not been specifically targeted in these therapies, careful monitoring of renal function with a particular emphasis on the glomerular filtration barrier should be included in the clinical protocols.

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Structure, Expression and Receptor-Binding Properties of Novel Vascular Endothelial Growth Factors

U. Eriksson[1] and K. Alitalo[2]

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1 Introduction

Vascular endothelial growth factor (VEGF), an important regulator of endothelial cell physiology, was identified some 10 years ago and has, since then, been recognised as the major growth factor relatively specific for endothelial cells (reviewed in Ferrara and Davis-Smyth 1997). VEGF is a dimeric glycoprotein, closely related to placenta growth factor (PlGF). Both VEGF and PlGF are distantly related in structure to the platelet-derived growth factors A and B (PDGF A and PDGF B) (Heldin et al. 1993). Three novel growth factors belonging to the family of VEGF, PlGF and the two PDGFs were recently discovered. These growth factors, termed vascular endothelial growth factor B/VEGF-related factor (VEGF-B/VRF) (Grimmond et al. 1996; Olofsson et al. 1996a), vascular endothelial growth factor C/VEGF-related protein (VEGF-C/VRP) (Joukov et al. 1996; Lee et al. 1996)] and *c-fos*-induced growth factor (FIGF) (Orlandini et al. 1996) share structural features typical of the VEGF/PDGF growth factor family. The prominent structural similarities between VEGF-related growth factors, several of which target endothelial cells, and FIGF suggest the possibility that FIGF also targets endothelial cells, despite its identification as a fibroblast growth factor. Based on these criteria,

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we propose that the name FIGF should be changed to VEGF-D to indicate its structural and functional relatedness to the other VEGFs.

The rapidly expanding list of growth factors belonging to the VEGF-family is surprising, but underscores the complexity of regulation of endothelial cell functions and the heterogeneity among different subpopulations of endothelial cells. In this review, we will summarise known structural and functional properties of the novel VEGFs, i.e. VEGF-B, VEGF-C and VEGF-D.

2 Identification and Properties of VEGF-B/VRF

A serendipitously found partial, mouse complementary deoxyribonucleic acid (cDNA) clone, encoding a VEGF-related peptide, was used to isolate full-length mouse and human cDNA clones from an adult mouse-heart cDNA library and from a human tumour cell cDNA library, respectively (Olofsson et al. 1996a). The full-length cDNAs encoded a homologue of VEGF and, in analogy with the nomenclature of the PDGFs, the new protein was denoted VEGF-B. Independently, another group of researchers found the same gene when attempting to identify the gene for multiple endocrine neoplasia type 1 (MEN1). The protein encoded by this gene was designated VEGF-related factor (VRF, Grimmond et al. 1996).

The mouse and human genes for VEGF-B are almost identical, and both span about 4 kb of DNA. The genes are composed of seven exons and their exon–intron organisation resembles that of the VEGF and PlGF genes (Fig. 1) (Grimmond et al. 1996; Olofsson et al. 1996b; Townson et al. 1996). Presently, two isoforms of VEGF-B, generated by alternative splicing of mRNA, have been recognised (Grimmond et al. 1996; Olofsson et al. 1996b; Townson et al. 1996). These two secreted forms of VEGF-B have 167 (VEGF-B_{167}) and 186 (VEGF-B_{186}) amino acid residues, respectively. The isoforms have an identical *N*-terminal domain of 115 amino acid residues, excluding the signal sequence, while the *C*-terminal domains differ. The common *N*-terminal domain is encoded by exons 1–5. Differential use of the remaining three exons gives rise to the two splice isoforms. By the use of an alternative splice-acceptor site in exon 6, an insertion of 101 bp introduces a frame shift and a stop of the coding region of VEGF-B_{167} cDNA (see Fig. 1). Thus, the two VEGF-B isoforms will have different *C*-terminal domains which are unrelated to each other. In VEGF and PlGF, several isoforms are encoded by the use of alternative splice-acceptor sites and different combinations of exons in the genes, but the corresponding transcripts are translated using the same reading frame. The use of partially overlapping, but different reading frames is fairly uncommon among higher eukaryotes.

The different *C*-terminal domains of the two splice isoforms of VEGF-B affect their biochemical and cell biological properties. The *C*-terminal domain of VEGF-B_{167} is structurally related to the corresponding region in VEGF, with several conserved cysteine residues and stretches of basic amino acid residues (see Sect. 5).

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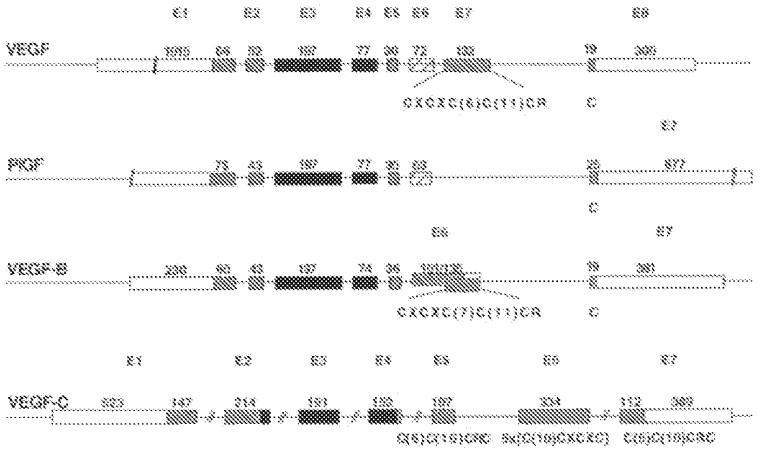


Fig. 1. Exon–intron organization of genes of the vascular endothelial growth factor (VEGF) family. The exons are shown as *boxes* and their lengths (bp) are indicated; the non-coding portions are *white*; *grey boxes* denote sequences encoding *C*- and *N*-terminal peptides; *black boxes* denote sequences encoding the VEGF homology domain. The *striped box* indicates the exon encoding part of the heparin-binding region. Certain cysteine motifs encoded by the different exons are shown. The structures of the genes are from Tischer et al. 1991, Olofsson et al. 1996b, Chilov et al. 1997 and DiPalma et al. 1997. The figure was modified from Chilov et al. 1997

Thus, this domain is highly hydrophilic and basic and, accordingly, VEGF-B_{167} will remain cell-associated on secretion, unless the producing cells are treated with heparin or high salt concentrations. The cell-associated molecules binding VEGF-B_{167} are likely to be cell surface or pericellular heparan sulphate proteoglycans. It is likely that the cell-association of this isoform occurs via its unique basic *C*-terminal region, as noted for the highly basic splice variants of VEGF. This suggestion is further supported by the observation that a fusion protein of glutathione-*S*-transferase and the unique *C*-terminal domain of VEGF-B_{167} binds tightly to a heparin-Sepharose column (B. Olofsson and the authors, unpublished observation).

The *C*-terminal domain of the second splice isoform, VEGF-B_{186}, has no significant similarity with known amino acid sequences in the databases. The hydrophobic character of this domain, with several conserved alanine, proline, serine and threonine amino acid residues contrasts with the properties of the hydrophilic and basic *C*-terminal domain in VEGF-B_{167}. This is supported by the observation that VEGF-B_{186} does not remain cell-associated on its secretion. Recent evidence suggests that this isoform is proteolytically processed, which regulates the biological properties of the protein (Olofsson et al. 1998 and unpublished data).

Isoforms of both human and mouse VEGF-B lack the consensus sequence for *N*-linked glycosylation (NXT/S), unlike the other growth factors of the PDGF/VEGF-family. However, VEGF-B_{186} is *O*-glycosylated, presumably in the unique *C*-terminal domain rich in serine and threonine residues (Olofsson et al. 1996b).

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The VEGF-B isoforms are produced as disulphide-linked homodimers and, under reducing conditions, the apparent molecular masses of secreted VEGF-B_{167} and VEGF-B_{186} isoforms are 21 kDa and 32 kDa, respectively (OLOFSSON et al. 1996a,b). The secreted 32 kDa form of VEGF-B_{186} is *O*-glycosylated, while the unmodified intracellular form of VEGF-B_{186} has an apparent molecular mass of 26kDa.

It is well documented that VEGF can form naturally occurring heterodimers with PlGF (DISALVO et al. 1995) and such heterodimers might display functional properties distinct from those of both VEGF and PlGF homodimers. Analysis of both isoforms of VEGF-B showed that disulphide-linked heterodimers with VEGF are generated when both are co-expressed in recipient cells (OLOFSSON et al. 1996a,b), but it has not been established whether naturally occurring VEGF–VEGF-B heterodimers exist. Homodimers of $VEGF_{165}$ are secreted from cells in a soluble form, while heterodimers of VEGF-B_{167}–VEGF remain cell-associated. In contrast, heterodimers of VEGF-B_{186} and VEGF are freely secreted into the cell-culture medium. Thus, VEGF-B_{167} appears to determine the release of the heterodimers from cells, and heterodimerization of VEGF with either of the two isoforms of VEGF-B might, therefore, control the release and bioavailability of VEGF–VEGF-B heterodimers (OLOFSSON et al. 1996a,b). It is presently unknown whether the VEGF-B polypeptides perform their in vivo function as homodimers, as heterodimers with VEGF, or as both.

The ability of VEGF-B isoforms to affect the release of VEGF–VEGF-B heterodimers from the producing cells is intriguing, since the two growth factors are co-expressed in many tissues, most prominently in the heart (OLOFSSON et al. 1996a). The almost identical patterns of expression of the two VEGF-B isoforms, predominantly in embryonic and adult muscle tissues (myocardium and skeletal muscle), makes it unlikely that differential expression of VEGF-B isoforms would contribute to a genetically controlled mechanism involved in the release of VEGF–VEGF-B heterodimers.

Conditioned medium from 293 cells transfected with an expression vector generating VEGF-B_{167} stimulated thymidine incorporation into DNA in human umbilical vein endothelial cells (HUVECs) and bovine capillary endothelial (BCE) cells. This suggested that VEGF-B is an endothelial cell mitogen and that it may be angiogenic in vivo (OLOFSSON et al. 1996a). However, the possibility remains that at least part of the mitogenic activity is contributed by VEGF–VEGF-B heterodimers, as recombinant VEGF-B_{186} homodimers have no detectable mitogenic activity on endothelial cells (OLOFSSON et al. 1998).

Despite their close structural similarities, the receptor-binding properties of VEGF-B differ from those of VEGF. Using soluble VEGF receptor (VEGFR) extracellular-domain fusion proteins, we have established that VEGF-B binds to VEGFR-1 with high affinity, but not to VEGFR-2 or -3 (OLOFSSON et al. 1998). The affinity for VEGFR-1 and not VEGFR-2 is not surprising, considering that certain receptor-specific epitopes defined for VEGF (KEYT et al. 1996) predict this reactivity. Thus, the acidic residues in loop 2 of VEGF, important for binding to VEGFR-1, are almost identical in VEGF-B. However, analysis of several VEGF-B

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mutants in which these acidic residues have been replaced by alanine residues show that the corresponding residues of VEGF-B has some effect for VEGFR-1 binding (Olofsson et al. 1998). Conversely, the basic residues in loop 3 of VEGF, important for VEGFR-2 binding, are not present in VEGF-B. The selective binding of VEGF-B and PlGF to VEGFR-1 suggests that the two growth factors may be differentially expressed functional homologues.

The expression of VEGF-B during most of murine development suggests that VEGF-B has a role during the establishment of the vascular system. Results from the knockout studies of VEGF have shown that VEGF-B is unable to compensate for the loss of even a single allele of VEGF (Carmeliet et al. 1996; Ferrara et al. 1996). Given that VEGF-B does not bind VEGFR-2, this is not surprising, and the functions of VEGF and VEGF-B are, thus, clearly distinct. Furthermore, the role of VEGF-B may extend beyond the vascular system as it is expressed early during the development of the central nervous system. VEGF-B expression was detected in 8-day-old embryos in structures most likely corresponding to parts of the neural tube (Lagercrantz et al. 1996). On day 11.5–12.5 p.c., VEGF-B was strongly expressed in the developing heart (Olofsson et al. 1996a and unpublished observations), Later, on day 14 p.c., VEGF-B is expressed in most tissues of the embryo, although most prominently in heart, spinal cord and cerebral cortex. On day 17, most of the in situ hybridization signal is concentrated in the heart, brown fat and spinal cord (Lagercrantz et al. 1996).

One of the unique features of VEGF expression is its upregulation under hypoxic conditions (Goldberg and Schneider 1994; Stein et al. 1995) and by a variety of other stimuli, including several growth factors and cytokines (Finkenzeller et al. 1992; Garrido et al. 1993; Pertovaara et al. 1994; Frank et al. 1995; Cohen et al. 1996). The regulation of VEGF-B mRNA is apparently very different, as neither hypoxia nor several growth factors alter the level of expression of this gene (Enholm et al. 1997).

The VEGF-B gene was localised to chromosome 11q13, proximal to the cyclin D1 gene, which is amplified in a number of human carcinomas (Paavonen et al. 1996). The amplification of cyclin D1, however, was not accompanied by amplification of VEGF-B in several mammary carcinoma cell lines studied (Paavonen et al. 1996).

3 Identification and Properties of VEGF-C/VRP

A factor stimulating tyrosine phosphorylation of Flt4 (subsequently referred to as VEGFR-3), a receptor tyrosine kinase closely related to VEGFR-1 and VEGFR-2, was identified in conditioned medium from PC-3 prostatic adenocarcinoma cells. Receptor-affinity chromatography using the VEGFR-3 extracellular domain led to the purification of the stimulating factor. The partial amino acid sequence was obtained from the purified factor and a 5′ fragment of the cDNA encoding it was

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amplified by serial polymerase chain reactions (PCR) using degenerate primers. A full-length cDNA was then cloned from a library prepared from PC-3 cells, using the labelled PCR-amplified 5′ fragment as a probe (Joukov et al. 1996). The full-length cDNA encoded a novel homologue of VEGF and was subsequently denoted VEGF-C. Independently, an expressed sequence tag (EST) was identified in the database as being homologous with VEGF. Using the partial EST clone as the probe, a full-length VEGF-C cDNA clone was isolated. The protein encoded by this cDNA was designated VEGF-related protein (VRP) (Lee et al. 1996).

The human VEGF-C cDNA encodes a protein of 419 amino acid residues, with a predicted molecular mass of 46.9 kDa. However, the newly synthesised VEGF-C product is a pre-pro-protein, consisting of an *N*-terminal signal sequence followed by an *N*-terminal peptide, the VEGF-homology domain, and a *C*-terminal pro-peptide (Joukov et al. 1996). VEGF-C is secreted as a disulphide bonded homodimer, and most of it is proteolytically processed from the precursor polypeptide, which contains three putative *N*-glycosylation sites; two of these remain in mature, fully processed VEGF homology domain. Based on our results, we propose the VEGF-C proteolytic processing model schematically presented in Fig. 2. This model resembles the model for the proteolytic processing of PDGF, especially of PDGF-B (Östman et al. 1988, 1992) in that: (1) the proteolytic cleavages occur after the formation of disulphide-bonded precursor dimers, (2) both *N*- and *C*-terminal peptides may be subject to cleavage, and (3) a variety of processed forms are secreted. However, there are several important differences between PDGF-B and VEGF-C, concerning both their processing and the structures of the mature growth factors.

The homologous part of VEGF-C is about 30% identical with $VEGF_{165}$, 27% with $VEGF\text{-}B_{167}$, 25% with PlGF-1 and 22–24% with PDGF-A and PDGF-B. Fully processed VEGF-C binds to and activates both VEGFR-3 and VEGFR-2. A single class of high-affinity sites was observed in porcine aortic endothelial (PAE)/VEGFR-3 cells ($K_d = 135$ pM) and PAE/VEGFR-2 cells ($K_d = 410$ pM). These values are of similar magnitude to the affinities reported for the VEGF–VEGFR-2 interaction (Terman et al. 1992; Waltenberger et al. 1994). VEGF-C and VEGF displace each other from VEGFR-2, indicating that the same region of this receptor is involved in the binding of both ligands. Surprisingly, none of the three basic residues reported to be critical for VEGFR-2 binding by VEGF (Keyt et al. 1996) are conserved in VEGF-C. VEGF-C also dose-dependently stimulated autophosphorylation of VEGFR-3 and VEGFR-2, but in agreement with previous reports (Lee et al. 1996), we could not detect binding to VEGFR-1 (Joukov et al. 1996, 1997).

The human and mouse VEGF-C genes both comprise over 40 kb of genomic DNA and consist of seven exons, all containing coding sequences (Fig. 1). The VEGF-C gene was localised to human chromosome 4q34, close to the human aspartylglucosaminidase gene (Paavonen et al. 1996). The VEGF homology domain of VEGF-C is encoded by exons 3 and 4. Exons 5 and 7 encode cysteine-rich motifs of the type C(6)C(10)CRC, and exon 6 encodes C(10)CXCXC motifs typical of a silk protein (Chilov et al. 1997). The upstream promoter sequences contain

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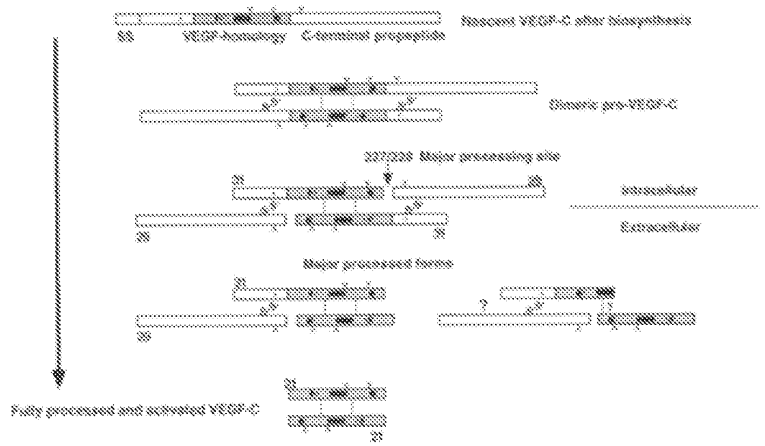


Fig. 2. Schematic model of the proteolytic processing of VEGF-C. The regions of VEGF-C polypeptide are marked as follows: *SS* signal sequence; *grey box* VEGF-homology domain; *open boxes N*-terminal and *C*-terminal peptides. Cysteine residues are shown as *black ovals*, the cysteine residues in the *C*-terminal pro-peptide are not marked for clarity. Putative sites of *N*-linked glycosylation are indicated by *Y*. The major proteolytic cleavage site between residues 227/228 is indicated by an *arrow*. This cleavage occurs as early as during secretion of the protein from the cellular compartment and it creates the major processed forms consisting of disulphide-linked 29/31-kDa polypeptides. Disulphide bonds are marked as *-S-S-*; the *dashed lines* indicate the detection of both covalent or non-covalent interactions. The proposed structure of the alternatively processed VEGF-C is indicated with a *question mark*. Several intermediate forms are omitted to simplify the scheme. The figure wad adopted from Joukov et al. (1997)

conserved putative binding sites for Sp-1, AP-2 and nuclear factor κB (NF-κB) transcription factors, but no TATA box, and show serum-stimulated promoter activity when transfected into cells. The VEGF-C gene structure is, thus, assembled from exons encoding pro-peptides and distinct cysteine-rich domains in addition to the VEGF homology domain, showing both similarities and distinct differences compared with other members of the VEGF/PDGF gene family.

VEGF-C mRNA was detected in Northern-blot analyses of many embryonal and adult tissues. In adult humans, the VEGF-C mRNA is expressed most prominently in heart, placenta, ovary, small intestine and the thyroid gland. Tumour cells express, almost exclusively, a 2.4-kb mRNA form, suggesting that it corresponds to the described VEGF-C cDNA clone obtained from the PC-3 tumour cell line (Joukov et al. 1996). The identity of another 2.0-kb mRNA, hybridizing with the VEGF-C probe in analysis of many tissues remains to be determined. Two VEGF-C cDNA clones were obtained that contained 152-bp and 557-bp deletions, corresponding to exon 2 or exons 2–4, respectively (Lee et al. 1996; Chilov et al. 1997) Due to the shift of the reading frame, which occurs 15 amino acid residues downstream of these deletions, the predicted proteins encoded by the two deleted cDNAs contain either no or only part of the core cysteine knot region similar to that in VEGF.

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Also the mouse VEGF-C cDNA was cloned and shown to encode a protein of 415 amino acid residues, which is 85% identical with human VEGF-C and similarly processed (Kukk et al. 1996). In in situ hybridization, mouse VEGF-C mRNA was detected in 8.5-day-old embryos in the cephalic mesenchyme, along the somites, in the tail region and extraembryonally in the allantois. In embryos 12.5 days p.c., VEGF-C mRNA was particularly prominent in regions where the lymphatic vessels are generated from embryonic veins, such as perimetanephric, axillary and jugular areas. The signal was also detected between the developing vertebrae, in the lung mesenchyme, in the neck region and in the developing forehead. The developing mesenterium, which is rich in lymphatic vessels, also showed strong VEGF-C expression (Kukk et al. 1996). The distribution of the VEGFR-3 mRNA follows a somewhat similar temporal and spatial pattern (Kaipainen et al. 1995; Kukk et al. 1996). This suggests a paracrine mode of ligand–receptor interaction, with VEGF-C expressed in mesenchymal cells adjacent to the VEGFR-3 positive endothelia. – The juxtaposed VEGFR-3 and VEGF-C expression patterns suggest that VEGF-C functions in the formation of the venous and lymphatic vascular systems during embryogenesis. Constitutive expression of VEGF-C in adult tissues further suggests that this growth factor is also involved in the maintenance of functions of, for example, differentiated lymphatic endothelium, where VEGFR-3 is expressed (Kaipainen et al. 1993, 1995; Kukk et al. 1996).

VEGF-C expression was detected in embryos as early as day 7 p.c. (Kukk et al. 1996). This was striking, considering the appearance of VEGFR-3 mRNA first on day 8.5 of gestation (Kaipainen et al. 1995). This suggests a possible role of VEGF-C during earlier stages of embryonic development. Such a function might be exercised through the ability of VEGF-C to function as a ligand for VEGFR-2, which is expressed in presumptive progenitors of yolk-sac blood islands as early as day 7 p.c. Interestingly, VEGFR-2 is essential for the development of both haematopoietic and endothelial cell lineages (Carmeliet et al. 1996; Ferrara et al. 1996). We, therefore, investigated the effect of VEGF-C on VEGFR-2 positive cells isolated from the primitive streak of gastrulating quail embryos. VEGF binding triggers endothelial differentiation of these cells, whereas haemopoietic differentiation appears to be mediated by binding of a so-far unidentified VEGFR-2 ligand. We could show that, like VEGF (Eichmann et al. 1997), VEGF-C also triggers endothelial differentiation of these cells, presumably via VEGFR-2 (Eichmann et al. 1998). These results indicate that VEGF and VEGF-C can act in a redundant manner via VEGFR-2.

Our results demonstrate that proteolytic processing allows VEGF-C to bind to and activate VEGFR-2 and increases its affinity and activity towards VEGFR-3 (Joukov et al. 1997). The biosynthesis of VEGF-C as a precursor may prevent unwanted angiogenic effects via VEGFR-2 and allow VEGF-C to signal preferentially via VEGFR-3. In certain circumstances, proteolytic processing would release mature VEGF-C, which is able to signal via both VEGFR-3 and VEGFR-2. It is also possible that activation of both VEGFR-3 and VEGFR-2, either as homo- or as heterodimers, is necessary to elicit a complete biological response to VEGF-C. Similarly, heterodimers of VEGFR-1 and VEGFR-2 could be important for the

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biological activities of VEGF. In the case of VEGF-C, proteolytic processing might provide a regulatory mechanism that provides the possibility for fine tuning of the biological functions of this growth factor.

The major secreted VEGF-C form contains the *C*-terminal pro-peptide, which has an unusual structure with tandemly repeated cysteine-rich motifs and is linked via disulphide bonds with the *N*-terminal peptide. The possible function of this, apparently in itself an inactive *C*-terminal half of VEGF-C, is unknown. It has domains of striking similarity to a secretory silk protein and contains short motifs homologous with the epidermal growth factor (EGF)-like domains of other secreted proteins, such as fibrillin, laminin and tenascin. All of these proteins are known to participate in protein–protein or protein–cell surface interactions. One can, thus, speculate that partially processed VEGF-C may stay associated with the extracellular matrix via its *C*-terminal pro-peptide (Fig. 2). Cleavage of the *N*-terminal pro-peptide in certain conditions by, as yet, unknown proteases would then release the active VEGF-C.

Like VEGF, VEGF-C also stimulates the migration of endothelial cells and increases vascular permeability, albeit at concentrations higher than required for VEGF (Joukov et al. 1996; Lee et al. 1996). About 50-fold higher concentrations of VEGF-C were required to induce the proliferation of blood vascular endothelial cells. These activities are probably mediated through VEGFR-2 activation (Park et al. 1994; Waltenberger et al. 1994). The lower specific activity of VEGF-C in these assays may depend on its lower affinity for VEGFR-2 and on its inability to bind VEGFR-1, precluding the formation of VEGFR-1–VEGFR-2 heterodimers, which may be required for maximal biological responses to VEGF (Waltenberger et al. 1994; DiSalvo et al. 1995; Cao et al. 1996; Clauss et al. 1996).

In order to better understand the function of VEGF-C, in vivo, its cDNA was expressed via a human keratin promoter in the basal cells of stratified squamous epithelia (Jeltsch et al. 1997). Histological examination of the transgenic mice showed that the dermis was atrophic and its connective tissue was replaced by large lymphatic vessels. In ultrastructural analysis, these vessels were shown to have overlapping endothelial junctions, anchoring filaments in the vessel wall, and a discontinuous or even partially absent basement membrane. The endothelium was also characterised by positive staining with monoclonal antibodies to desmoplakins I and II, expressed in lymphatic, but not in vascular, endothelial cells (Schmelz et al. 1994). VEGFR-3 and VEGFR-2, and the Tie-1 endothelial-receptor tyrosine kinase mRNAs were detected in endothelial cells lining the abnormal vessels. The VEGF-C–receptor interaction in transgenic mice apparently transduced a mitogenic signal because, in contrast to littermate controls, the lymphatic endothelium of the skin from young transgenic mice showed increased DNA synthesis. In fluorescent microlymphography, a typical honeycomb-like network with similar mesh sizes was detected in both control and transgenic mice, but the diameter of the vessels was approximately twice as large in the transgenic mice. Thus, the endothelial proliferation induced by VEGF-C led to hyperplasia of the superficial lymphatic network, but did not induce the sprouting of new vessels. Also, a relatively specific lymphangiogenic response was obtained when recombinant VEGF-

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C was applied to the differentiated chick chorioallantoic membrane (Oh et al. 1997).

These effects of VEGF-C overexpression were unexpectedly specific, particularly as VEGF-C is also capable of binding to and activating VEGFR-2 of blood vessel endothelial cells. In vivo, the specific effects of VEGF-C on lymphatic endothelial cells may reflect a requirement for the formation of VEGFR-3–VEGFR-2 heterodimers for endothelial cell proliferation. Such possible heterodimers may help to explain how three homologous VEGFs exert partially redundant, yet strikingly specific, biological effects. Thus, VEGF-C induces specific lymphatic endothelial proliferation and hyperplasia of the lymphatic vasculature in vivo. Further studies should establish the role of VEGF-C in lymphangiomas and in tumour metastasis via the lymphatic vasculature as well as in various other disorders involving the lymphatic system and their treatment.

Both VEGF and VEGF-C are potent vascular-permeability factors. Surprisingly, we have found that the recombinant mature VEGF-C, in which Cys156 was replaced by a Ser residue, is a selective agonist of VEGFR-3 (Joukov et al. 1998). This mutant, designated ΔNΔC156S, binds and activates VEGFR-3, but neither binds VEGFR-2 nor activates its autophosphorylation and downstream signalling to the ERK/MAPK pathway. Unlike VEGF-C, ΔNΔC156S neither induces vascular permeability in vivo nor stimulates migration of bovine capillary endothelial cells in culture. These data point out the critical role of VEGFR-2-mediated signal transduction for the vascular permeability activity of VEGF-C, and strongly suggest that the redundancy of biological effects of VEGF and VEGF-C is caused by their ability to bind to and activate VEGFR-2. However, the possibility exists that there are additional receptors for VEGF and VEGF-C, that are responsible for vascular permeability. The ΔNΔC156S mutant may provide a valuable tool for the analysis of VEGF-C effects mediated selectively via VEGFR-3. The ability of ΔNΔC156 S to form homodimers also emphasises differences in the structural requirements for VEGF and VEGF-C dimerization.

Serum and its component growth factors, PDGF, EGF and transforming growth factor-β (TGF-β), and tumor promoters were found to stimulate VEGF-C, but not VEGF-B, mRNA expression (Enholm et al. 1997). Serum induction of VEGF-C mRNA occurred independently of protein synthesis; with a slight increase of the mRNA half-life, whereas VEGF-B mRNA was very stable. Hovewer, hypoxia, *Ras* oncoprotein and mutant p53 tumour suppressor, which are potent inducers of VEGF mRNA did not increase VEGF-B or VEGF-C mRNA levels. We have also studied the regulation of VEGF-C by angiogenic pro-inflammatory cytokines. Interleukin (IL)-1 induced a concentration- and a time-dependent increase in VEGF-C, but not in VEGF-B, mRNA steady-state levels in human lung fibroblasts, mainly due to increased transcription (Ristimaki et al. 1998). Tumour necrosis factor alpha (TNFα) and IL-1 also elevated VEGF-C mRNA steady-state levels, whereas the IL-1 receptor antagonist and dexamethasone inhibited the effect of IL-1. Hypoxia, which is an important inducer of VEGF expression, had no effect on VEGF-B or VEGF-C mRNA levels (Enholm et al. 1997). IL-1 and TNFα also stimulated the production of VEGF-C protein by the fibroblasts (Ristimaki et al.

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1998). Our data suggest that in addition to VEGF, VEGF-C may also serve as a lymphangiogenic or angiogenic stimulus at sites of cytokine activation. In particular, these results raise the possibility that certain pro-inflammatory cytokines regulate the lymphatic vessels indirectly via VEGF-C.

4 Identification and Properties of VEGF-D/FIGF

A partial cDNA for FIGF was first isolated from a differential-display screening of murine fibroblast mRNAs from cells with or without a targeted inactivation of the *c-fos* locus (ORLANDINI et al. 1996). The full-length murine cDNA clone was found to encode a protein of 358 amino acid residues, including a hydrophobic putative signal sequence, with significant similarities to the PDGF/VEGF family of growth factors (see Sect. 5). FIGF was shown to stimulate mitosis of fibroblasts in a dose-dependent manner. However, based on the strong structural similarities to the PDGF/VEGF family of growth factors, we propose that FIGF should be renamed to VEGF-D to highlight this relationship and to get a rational nomenclature of the novel VEGFs. VEGF-D can be viewed as having a VEGF homology domain and long *N*- and *C*-terminal extensions. The fact that VEGF-D is most closely related to VEGF-C is apparent for two reasons (see Sect. 5): first, the VEGF homology domain of VEGF-D is much more closely related to that found in VEGF-C than to those of the other family members; second, of the other factors in the VEGF family, only VEGF-C has long *N*- and *C*-terminal extensions similar to those in VEGF-D. The presence of these extensions in VEGF-C and VEGF-D, thus, defines a new subfamily of the VEGFs.

The similarity between VEGF-D and VEGF-C exists also at the functional level, as receptor-binding studies demonstrated that VEGF-D and VEGF-C exhibit similar receptor specificities (ACHEN et al. 1998). This protein is likely to be processed in a similar fashion to VEGF-C (data not shown). A region of VEGF-D corresponding to the fully processed, mature VEGF-C can bind to the extracellular domain of VEGFR-2 and induce tyrosine phosphorylation of both VEGFR-2 and VEGFR-3. When expressed in insect cells, the full-length VEGF-D was not proteolytically processed; this protein was unable to activate VEGFR-3 and activation of VEGFR-2 was, at best, marginal. Given that VEGF-D can also activate VEGFR-3, it is possible that VEGF-D could be involved in the regulation of the growth and/or differentiation of lymphatic endothelium just like VEGF-C.

The notion that VEGF-D and VEGF-C may have similar biological functions is further supported by their similar expression patterns. For example, both genes are strongly expressed in heart, muscle and small intestine, whereas expression was undetectable in peripheral blood leucocytes, brain and liver (JOUKOV et al. 1996; LEE et al. 1996; ACHEN et al. 1998). Nevertheless, the expression patterns are not identical. A second VEGF-D transcript was detected only in skeletal muscle.

Like VEGF-C, human VEGF-D was also mitogenic for bovine aortic endothelial cells. This response is likely to involve VEGFR-2. Mouse VEGF-D has

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previously been reported to induce proliferation and morphological alterations of cultured fibroblasts, but the receptors responsible for mediating these effects have not been identified (ORLANDINI et al. 1996). It would be of interest to determine whether or not the cultured fibroblasts used for such studies expressed VEGFR-2 or VEGFR-3. A summary of the receptor-binding properties of known members of the VEGF family of growth factors is illustrated in Fig. 3.

5 Primary Structures of VEGF-Related Growth Factors

Seven polypeptides with significant similarities to VEGF and two PDGFs have been discovered so far. A multiple amino acid sequence alignment of human VEGF, PlGF, VEGF-B, VEGF-C, VEGF-D and the two PDGFs show that a central core of the proteins is well conserved during evolution (Fig. 4A). A major part of this core region is located between the eight invariant cysteine residues, shown to be involved in inter- and intramolecular disulphide bonding of VEGF and the two PDGFs. This region is encoded by the two well-conserved exons, E3 and E4, in the corresponding genes of the VEGFs (see Fig. 1). The overall amino acid sequence identity in this region varies between 20% and 56% in pairwise comparisons of available amino acid residues. Outside this central core, the overall amino acid identities are much weaker, although individual pairs of these proteins display higher sequence similarities. The receptor binding epitopes in these group of growth factors, at least for VEGF (KEYT et al. 1996) and the two PDGFs (HELDIN et al. 1993), are confined within the central core defined by the eight invariant cysteine residues. Thus, this region defines a structural and functional minimal domain. Determination of the three dimensional structures of VEGF (MULLER

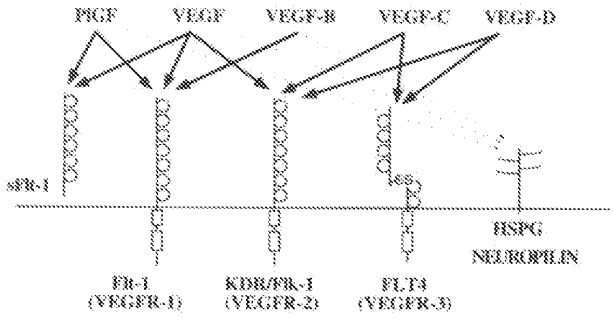
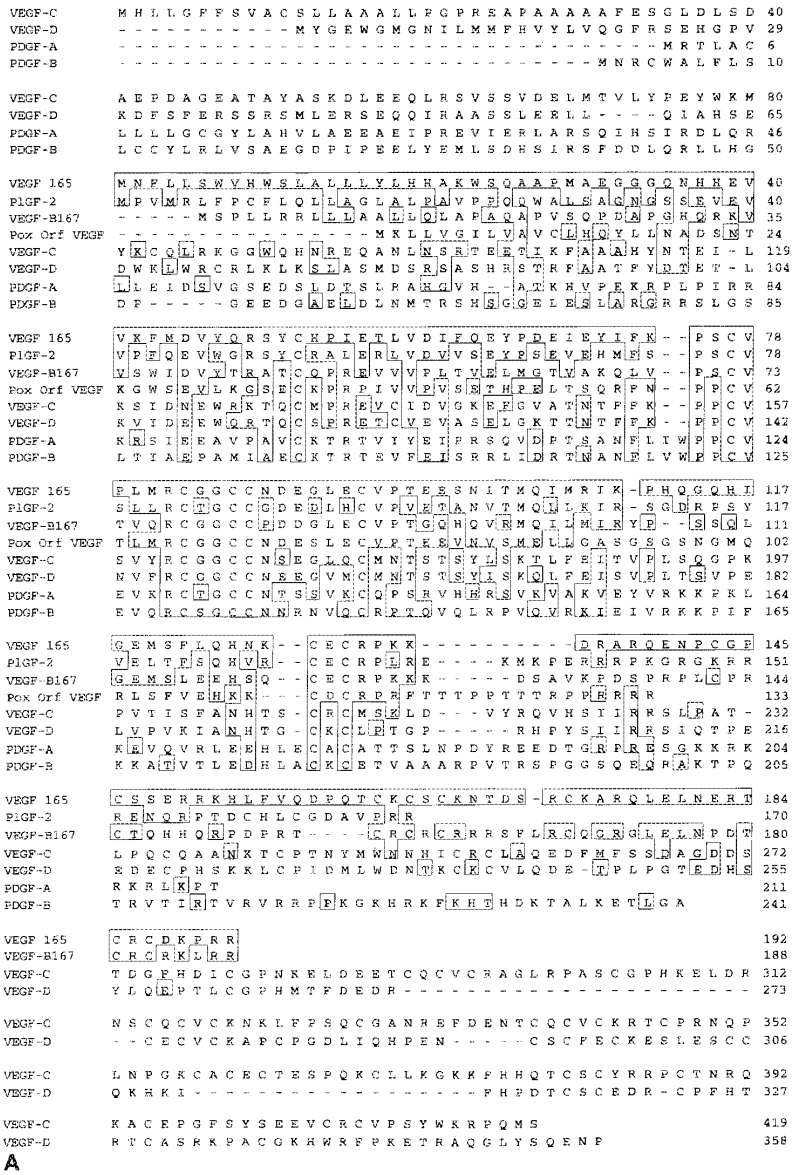


Fig. 3. Interactions of VEGF-related growth factor with their receptors. A schematic illustration of the receptor-binding characteristics of VEGF-related growth factors with soluble Flt-1 (sFlt-1), Flt-1 (*VEGFR-1*), KDR/Flk-1 (*VEGFR-2*), Flt-4 (*VEGFR-3*) and with heparin sulphate proteoglycans (*HSPG*) and neuropilin (SOKER et al. 1998)

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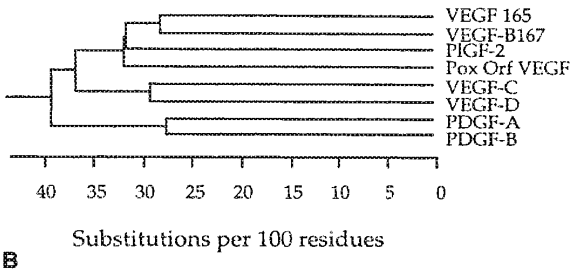


Fig. 4A, B. Amino acid sequence alignment of VEGF-related growth factors. **A**. Multiple amino acid sequence alignment of three novel VEGFs, e.g. VEGF-B, VEGF-C and VEGF-D and a comparison with VEGF, placenta growth factor (PlGF) and the two platelet-derived growth factors (PDGFs) as well as with the interesting viral homologue of the poxvirus *orf* virus (LYTTLE et al. 1994). The amino acid sequences were aligned using the Crystal algorithm, and the alignment was refined manually. The *boxed* residues are within two distance units using the PAM 250 matrix. **B**. An unrooted phylogenetic tree of the VEGF-related growth factors based on the amino acid sequence alignment in A

et al. 1997) and PDGF-B (OEFNER et al. 1992) by X-ray crystallography have also shown that this region forms distinct domains with remarkable structural similarities.

A phylogenetic analysis of the amino acid sequences of these growth factors shows that they can be grouped into three separate subfamilies, consisting of VEGF, PlGF, VEGF-B, and VEGF-C and D, and the two PDGFs, respectively (Fig. 4B).

6 Perspectives

The discovery of three novel members of VEGF family increases our understanding of the complexity of the regulatory signals for endothelial cells and promotes new areas of research in vascular biology. Many of the already-established experimental models and approaches used in VEGF studies might obviously be applied to studies of the novel VEGFs. However, not only endothelial functions should be taken into consideration here, as recent results show that VEGF might induce certain biological effects via the targeting of non-endothelial cells (MIDY and PLOUET 1994; GABRILOVICH et al. 1996).

The main questions regarding the biological roles of the novel VEGFs are not answered yet. In this regard, different molecular genetic and transgenic approaches, including gene targeting, are of great importance. Studies on VEGF-C have shown that it acts as a specific growth factor for endothelial cells of lymphatic vessels (JELTSCH et al. 1997). Studies on VEGF-B and VEGF-D are likely to provide additional information on the role of these growth factors in endothelial cell function and physiology.

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Important issues also concern the analysis of tissue-specific regulation of VEGF-B, VEGF-C and VEGF-D expression by hypoxia, various growth factors and other agents or conditions known to regulate VEGF expression. Similarly, the function of the different splicing forms of VEGF-B, VEGF-C and VEGF-D should be explored. Such alternatively spliced isoforms of these growth factors might possess different functions in vivo, e.g. due to the differences in their receptor specificity/affinity, bioavailability, stability and proteolytic processing, and their ability to form heterodimers with other VEGF family members. The latter property might be of particular importance, as heterodimers of the various growth factors might express biological properties distinct from those of the corresponding homodimers. Finally, the discovery of VEGF-B, VEGF-C and VEGF-D highlights the structural similarities of VEGF family polypeptides and may simplify the search for novel homologous molecules.

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N. FERRARA

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1 Introduction

The development of a vascular supply is a fundamental requirement for organ development and differentiation during embryogenesis as well as for wound healing and reproductive functions in the adult (FOLKMAN 1995). Angiogenesis is also implicated in the pathogenesis of a variety of disorders: proliferative retinopathies,

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age-related macular degeneration, tumors, rheumatoid arthritis and psoriasis (FOLKMAN 1995; GARNER 1994).

The search for positive regulators of angiogenesis has yielded several candidates, including fibroblast growth factors a and b (aFGF, bFGF), transforming growth factors alpha and beta (TGF-α, TGF-β), hepatocyte growth factor (HGF), tumor necrosis factor alpha (TNF-α), angiogenin, interleukin-8 (IL-8), etc. (FOLKMAN and SHING 1992; RISAU 1997). However, in spite of extensive research, there is still significant debate as to their role as endogenous mediators of angiogenesis. The negative regulators identified so far include thrombospondin (GOOD et al. 1990; DIPIETRO 1997), the 16-kilodalton *N*-terminal fragment of prolactin (FERRARA et al. 1991), angiostatin (O'REILLY et al. 1994) and endostatin (O'REILLY et al. 1997).

This chapter discusses the molecular and biological properties of the vascular endothelial growth factor (VEGF) proteins. Over the last few years, several additional members of the VEGF gene family have been identified, including VEGF-B, VEGF-C, Placenta growth factor (PlGF) and VEGF-D. This chapter focuses primarily on VEGF, also referred to as "VEGF-A". For a description of the other members of the family, the reader is referred to the appropriate chapters in this book. Work done by several laboratories over the last few years has elucidated the pivotal role of VEGF and its receptors in the regulation of normal and abnormal angiogenesis (FERRARA and DAVIS-SMYTH 1997). The finding that the loss of even a single VEGF allele results in embryonic lethality points to an irreplaceable role played by this factor in the development and differentiation of the vascular system (FERRARA et al. 1996; CARMELIET et al. 1996). Furthermore, VEGF-induced angiogenesis has been shown to result in a therapeutic effect in animal models of coronary or limb ischemia and, most recently, in a human patient affected by critical leg ischemia (FERRARA and DAVIS-SMYTH 1997).

2 Biological Activities of Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is a mitogen for vascular endothelial cells derived from arteries, veins and lymphatics, but is devoid of consistent and appreciable mitogenic activity for other cell types (FERRARA and DAVIS-SMYTH 1997). VEGF promotes angiogenesis in tri-dimensional in vitro models, inducing confluent microvascular endothelial cells to invade collagen gels and form capillary-like structures (PEPPER et al. 1992). Also, VEGF induces sprouting from rat aortic rings embedded in a collagen gel (NICOSIA et al. 1994). VEGF also elicits a pronounced angiogenic response in a variety of in vivo models, including the chick chorioallantoic membrane (LEUNG et al. 1989), the primate iris (TOLENTINO et al. 1996) etc.

VEGF induces expression of the serine proteases urokinase-type and tissue-type plasminogen activators (PA), and also PA inhibitor 1 (PAI-1) in cultured

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bovine microvascular endothelial cells (PEPPER et al. 1991). Moreover, VEGF increases expression of the metalloproteinase interstitial collagenase in human umbilical-vein endothelial cells (HUVEC), but not in dermal fibroblasts (UNEMORI et al. 1992). Other studies have shown that VEGF promotes expression of the urokinase receptor (uPAR) in vascular endothelial cells (MANDRIOTA et al. 1995). Additionally, VEGF stimulates hexose transport in cultured vascular endothelial cells (PEKALA et al. 1990).

VEGF is known also as vascular permeability factor (VPF), based on its ability to induce vascular leakage in the guinea-pig skin (DVORAK et al. 1995). DVORAK and colleagues proposed that an increase in microvascular permeability is a crucial step in angiogenesis associated with tumors and wounds (DVORAK 1986). According to this hypothesis, a major function of VPF/VEGF in the angiogenic process is the induction of plasma-protein leakage. This effect would result in the formation of an extravascular fibrin gel, a substrate for endothelial and tumor cell growth (DVORAK et al. 1987). Recent studies have also suggested that VEGF may induce fenestrations in endothelial cells (ROBERTS and PALADE 1995, 1997). Topical administration of VEGF acutely resulted in the development of fenestrations in the endothelium of small venules and capillaries, even in regions where endothelial cells are not normally fenestrated, and was associated with increased vascular permeability (ROBERTS and PALADE 1995, 1997).

MELDER et al. (1996) have shown that VEGF promotes expression of VCAM-1 and ICAM-1 in endothelial cells. This induction results in the adhesion of activated natural killer (NK) cells to endothelial cells, mediated by specific interaction of endothelial VCAM-1 and ICAM-1 with CD18 and VLA-4 on the surface of NK cells.

VEGF has been reported to have certain regulatory effects on blood cells. CLAUSS et al. (1990) reported that VEGF may promote monocyte chemotaxis, while BROXMEYER et al. (1995) have shown that VEGF induces colony formation by mature subsets of granulocyte-macrophage progenitor cells. These findings may be explained by the common origin of endothelial and hematopoietic cells and the presence of VEGF receptors in progenitor cells as early as hemangioblasts in blood islands in the yolk sac. Furthermore, GABRILOVICH et al. (1996) have reported that VEGF may have an inhibitory effect on the maturation of host professional antigen-presenting cells, such as dendritic cells. VEGF was found to inhibit immature dendritic cells, without having a significant effect on the function of mature cells. These findings led to the suggestion that VEGF may also facilitate tumor growth by allowing the tumor to avoid the induction of an immune response (GABRILOVICH et al. 1996).

VEGF induces vasodilatation in vitro in a dose-dependent fashion (KU et al. 1993; YANG et al. 1996) and produces transient tachycardia, hypotension and a decrease in cardiac output when injected intravenously in conscious, instrumented rats (YANG et al. 1996). Such effects appear to be caused by a decrease in venous return, mediated primarily by endothelial cell-derived nitric oxide (NO), as assessed by the requirement for an intact endothelium and the prevention of the effects by *N*-methyl-arginine (YANG et al. 1996). Accordingly, VEGF has no direct effect on contractility or rate in the isolated rat heart in vitro (YANG et al. 1996). These

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hemodynamic effects, however, are not unique to VEGF: other angiogenic factors, such as aFGF and bFGF, also have the ability to induce NO-mediated vasodilatation and hypotension (CUEVAS et al. 1991, 1996).

3 Organization of the VEGF Gene and Characteristics of the VEGF Proteins

The human VEGF gene is organized in eight exons, separated by seven introns. The coding region spans approximately 14 kb (HOUCK et al. 1991; TISCHER et al. 1991). The human VEGF gene has been assigned to chromosome 6p21.3 (VINCENTI et al. 1996). It is now well established that alternative exon splicing of a single VEGF gene results in the generation of four different molecular species, having respectively 121, 165, 189 and 206 amino acids following signal sequence cleavage ($VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$). $VEGF_{165}$ lacks the residues encoded by exon 6, while $VEGF_{121}$ lacks the residues encoded by exons 6 and 7. Compared with $VEGF_{165}$, $VEGF_{121}$ lacks 44 amino acids; $VEGF_{189}$ has an insertion of 24 amino acids, highly enriched in basic residues; and $VEGF_{206}$ has an additional insertion of 17 amino acids (HOUCK et al. 1991). Analysis of the VEGF gene promoter region reveals a single major transcription start which lies near a cluster of potential Sp1 factor binding sites.

$VEGF_{165}$ is the predominant molecular species produced by a variety of normal and transformed cells. Transcripts encoding $VEGF_{121}$ and $VEGF_{189}$ are detected in the majority of cells and tissues expressing the VEGF gene (HOUCK et al. 1991). In contrast, $VEGF_{206}$ is a very rare form, so far identified only in a human fetal liver complementary deoxyribonucleic acid (cDNA) library (HOUCK et al. 1991). The genomic organization of the murine VEGF gene has been also described (SHIMA et al. 1996). Similarly to the human gene, the coding region of the murine VEGF gene encompasses approximately 14kb and is comprised of eight exons interrupted by seven introns. Analysis of exons suggests the generation of three isoforms: $VEGF_{120}$, $VEGF_{164}$ and $VEGF_{188}$. Therefore, murine VEGFs are shorter than human VEGF by one amino acid. However, a fourth isoform comparable with $VEGF_{206}$ is not predicted, since an in-frame stop codon is present the region corresponding to the human $VEGF_{206}$ open reading frame. Analysis of the 3′ untranslated region of the rat VEGF messenger ribonucleic acid (mRNA) has revealed the presence of four potential polyadenylation sites (LEVY et al. 1996). A frequently used site is about 1.9kb further downstream from the previously reported transcription termination codon (CONN et al. 1990). The sequence within this 3′ untranslated region reveals a number of sequence motifs that are known to be involved in the regulation of mRNA stability (LEVY et al. 1996).

Native VEGF is a basic, heparin-binding, homodimeric glycoprotein of 45,000Da (FERRARA and HENZEL 1989). These properties correspond to those of $VEGF_{165}$, the major isoform (HOUCK et al. 1992). $VEGF_{121}$ is a weakly acidic

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polypeptide that fails to bind to heparin (HOUCK et al. 1992). $VEGF_{189}$ and $VEGF_{206}$ are more basic and bind to heparin with a greater affinity than $VEGF_{165}$ (HOUCK et al. 1992). Such differences in the isoelectric point and the affinity for heparin may affect the bioavailability of VEGF profoundly.

$VEGF_{121}$ is a freely diffusible protein; $VEGF_{165}$ is also secreted, although a significant fraction remains bound to the cell surface and the extracellular matrix (ECM). In contrast, $VEGF_{189}$ and $VEGF_{206}$ are almost completely sequestered in the ECM (PARK et al. 1993). However, these isoforms may be released in a soluble form by heparin or heparinase, suggesting that their binding site is represented by proteoglycans containing heparin-like moieties. The long forms may also be released by plasmin following cleavage at the carboxy (COOH) terminus. This action generates a bioactive proteolytic fragment with a molecular weight of ~34,000Da (HOUCK et al. 1992).

Plasminogen activation and generation of plasmin have been shown to play an important role in the angiogenesis cascade. Thus, proteolysis of VEGF is likely also to occur in vivo. KEYT et al. (1996a) have shown that the bioactive product of plasmin action is comprised of the first 110 amino (NH_2)-terminal amino acids of VEGF. These findings suggest that the VEGF proteins may become available to endothelial cells by at least two different mechanisms: as freely diffusible proteins ($VEGF_{121}$, $VEGF_{165}$) or following protease activation and cleavage of the longer isoforms. However, loss of heparin binding, whether it is due to alternative splicing of RNA or plasmin cleavage, results in a substantial loss of mitogenic activity for vascular endothelial cells: compared with $VEGF_{165}$, $VEGF_{121}$ or $VEGF_{110}$ which demonstrate a 50- to 100-fold reduced potency when tested in endothelial cell growth assay (KEYT et al. 1996a).

It has been suggested that the stability of VEGF–heparan-sulfate-receptor complexes contributes to effective signal transduction and stimulation of endothelial cell proliferation (KEYT et al. 1996a). Thus, VEGF has the potential to express structural and functional heterogeneity to yield a graded and controlled biological response. Very recently, POLTORAK et al. (1997) provided evidence for the existence of an additional, alternatively spliced molecular species of VEGF. A VEGF isoform containing exons 1–6 and 8 of the VEGF gene was found to be expressed as a major VEGF mRNA form in several cell lines derived from carcinomas of the female reproductive system. This mRNA is predicted to encode a VEGF form of 145 amino acids ($VEGF_{145}$). Recombinant $VEGF_{145}$ induced the proliferation of vascular endothelial cells, albeit at much lower potency than $VEGF_{165}$. $VEGF_{145}$ binds to the kinase domain region (KDR) receptor, also denoted VEGF receptor-2 (VEGFR-2) on the surface of endothelial cells. It also binds to heparin with an affinity similar to that of $VEGF_{165}$.

Recently, MULLER et al. (1997) determined the crystal structure of VEGF at a resolution of 2.5A. Overall, the VEGF monomer resembles that of platelet-derived growth factor (PDGF), but its *N*-terminal segment is helical rather than extended. The dimerization mode of VEGF is similar to that of PDGF and very different from that of TGF-β. Functional analysis of the binding epitopes for two receptor-

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blocking antibodies reveal different binding determinants near each of the VEGFR-2 binding hot spots.

4 Regulation of VEGF Gene Expression

4.1 Oxygen Tension

Among the mechanisms that have been proposed to participate in the regulation of VEGF gene expression, oxygen tension plays a major role, both in vitro and in vivo. VEGF mRNA expression is rapidly and reversibly induced by exposure to low pO_2 in a variety of normal and transformed cultured-cell types (MINCHENKO et al. 1994; SHIMA et al. 1995). Also, ischemia caused by occlusion of the left anterior descending coronary artery results in a dramatic increase in VEGF RNA levels in the pig and rat myocardium, suggesting that VEGF may mediate the spontaneous revascularization that follows myocardial ischemia (BANAI et al. 1994b; HASHIMOTO et al. 1994). Furthermore, hypoxic upregulation of VEGF mRNA in neuroglial cells, secondary to the onset of neuronal activity, has been proposed to play an important physiological role in the development of the retinal vasculature (STONE et al. 1995).

Similarities exist between the mechanisms leading to hypoxic regulation of VEGF and erythropoietin (Epo) (GOLDBERG and SCHNEIDER 1994). Hypoxia-inducibility is conferred on both genes by homologous sequences. By deletion and mutation analysis, a 28-base sequence has been identified in the 5′ promoter of the rat and human VEGF gene, which mediates hypoxia-induced transcription (LEVY et al. 1995; LIU et al. 1995). Such a sequence reveals a high degree of homology and similar protein-binding characteristics as the hypoxia-inducible factor 1 (HIF-1) binding site, within the Epo gene (MADAN and CURTIN 1993). HIF-1 has been identified as a mediator of transcriptional responses to hypoxia and is a basic, heterodimeric, helix–loop–helix protein (WANG and SEMENZA 1995). When reporter constructs containing the VEGF sequences that mediate hypoxia-inducibility were co-transfected with expression vectors encoding HIF-1 subunits, reporter gene transcription was much greater than that observed in cells transfected with the reporter alone, both in hypoxic and normoxic conditions (FORSYTHE et al. 1996).

However, transcriptional activation is not the only mechanism leading to VEGF upregulation in response to hypoxia (IKEDA et al. 1995; LEVY et al. 1996). Increased mRNA stability has been identified as a significant post-transcriptional component. Sequences that mediate increased stability were identified in the 3′ untranslated region of the VEGF mRNA.

4.2 Cytokines

Various cytokines or growth factors may upregulate VEGF mRNA expression. Epidermal growth factor (EGF), TGF-β or keratinocyte growth factor (KGF)

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result in a marked induction of VEGF mRNA expression (FRANK et al. 1995) EGF also stimulates VEGF release by cultured glioblastoma cells (GOLDMAN et al. 1993). In addition, treatment of quiescent cultures of epithelial and fibroblastic cell lines with TGF-β resulted in induction of VEGF mRNA and release of VEGF protein in the medium (PERTOVAARA et al. 1994). Based on these findings, it has been proposed that VEGF may function as a paracrine mediator for indirectly acting angiogenic agents, such as TGF-β (PERTOVAARA et al. 1994). Furthermore, IL-1-β induces VEGF expression in aortic smooth muscle cells (LI et al. 1995). Both IL-1-α and prostaglandin E_2(PGE_2) have been shown to induce expression of VEGF in cultured synovial fibroblasts, suggesting the participation of such inductive mechanisms in inflammatory angiogenesis (BEN-AV et al. 1995). IL-6 has also been shown to significantly induce VEGF expression in several cell lines (COHEN et al. 1996). IGF-1, a mitogen implicated in the growth of several malignancies, has also been shown to induce VEGF mRNA and protein in cultured colorectal carcinoma cells (WARREN et al. 1996). Such induction was mediated by a combined increase in the transcriptional rate of the VEGF gene and in the stability of the mRNA.

4.3 Differentiation and Transformation

Cell differentiation has been shown to play an important role in the regulation of VEGF gene expression (CLAFFEY et al. 1992). The VEGF mRNA is upregulated during the conversion of 3T3 pre-adipocytes into adipocytes or during the myogenic differentiation of C2C12 cells. Conversely, VEGF gene expression is repressed during the differentiation of the pheochromocytoma cell line PC12 into non-malignant, neuron-like cells.

Specific transforming events also result in induction of VEGF gene expression. A mutated form of the murine p53 tumor-suppressor gene has been shown to result in induction of VEGF mRNA expression in NIH 3T3 cells in transient transfection assays (KIESER et al. 1994). Likewise, oncogenic mutations or amplification of *ras* lead to VEGF upregulation (RAK et al. 1995; GRUGEL et al. 1995). Interestingly, expression of oncogenic *ras*, either constitutive or transient, potentiated the induction of VEGF by hypoxia (MAZURE et al. 1996). Moreover, the von Hippel-Lindau (VHL) tumor-suppressor gene has been recently implicated in the regulation of VEGF gene expression (SIEMEISTER et al. 1996; ILIOPOULOS et al. 1996; GNARRA et al. 1996).

The VHL tumor-suppressor gene is inactivated in patients with VHL disease and in most sporadic clear cell renal carcinomas. Although the function of the VHL protein remains to be fully elucidated, it is known that such a protein interacts with the elongin BC subunits in vivo, and regulates RNA polymerase-II elongation activity in vitro by inhibiting formation of the elongin ABC complex. Human renal cell carcinoma cells, either lacking endogenous wild-type VHL gene or expressing an inactive mutant, demonstrated altered regulation of VEGF gene expression, which was corrected by introduction of the wild-type VHL gene. Most of the endothelial cells' mitogenic activity released by tumor cells expressing the mutant

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VHL gene was neutralized by anti-VEGF antibodies (SIEMEISTER et al. 1996). These findings suggest that VEGF is a key mediator of the abnormal vascular proliferations and solid tumors characteristic of VHL syndrome.

ILIOPULOS et al. (1996) have shown that one function of the VHL protein is to provide a negative regulation of a series of hypoxia-inducible genes, including the VEGF, PDGF-B chain and the glucose transporter GLUT1 genes. In the presence of a mutant VHL, mRNAs for such genes were produced under both normoxic and hypoxic conditions. Reintroduction of wild-type VHL resulted in inhibition of mRNA production under normoxic conditions and restored the characteristic hypoxia-inducibility of those genes (ILIOPOULOS et al. 1996). In addition, GNARRA et al. (1996) have shown that VHL regulates VEGF expression at a post-transcriptional level and that VHL inactivation in target cells causes a loss of VEGF suppression, leading to formation of a vascular stroma. Interestingly, despite fivefold differences in VEGF mRNA levels, VHL overexpression did not affect VEGF transcription initiation.

5 The VEGF Receptors

Two classes of high-affinity VEGF binding sites on the surface of bovine endothelial cells were described initially, with K_d values of 10 pM and 100 pM (VAISMAN et al. 1990; PLOUET and MOUKADIRI 1990). Lower affinity binding sites on mononuclear phagocytes were subsequently described (SHEN et al. 1993). It has been suggested that such binding sites are involved in mediating the chemotactic effects of VEGF for monocytes (CLAUSS et al. 1990).

Ligand autoradiography studies on fetal and adult rat tissue sections demonstrated that high-affinity VEGF binding sites are localized to the vascular endothelium of large or small vessels in situ (JAKEMAN et al. 1992, 1993). VEGF binding was apparent not only on proliferating, but also on quiescent endothelial cells (JAKEMAN et al. 1992, 1993). Also, the earliest developmental identification of high-affinity VEGF binding was in hemangioblasts in the blood islands in the yolk sac (JAKEMAN et al. 1993).

6 The VEGFR-1 and VEGFR-2 Tyrosine Kinases

6.1 Binding Characteristics

Two VEGF receptor tyrosine kinases (RTKs) have been identified. The VEGFR-1 (fms-like-tyrosine kinase) (DE VRIES et al. 1992) and VEGFR-2 (KDR; TERMAN et al., 1992) receptors bind VEGF with high affinity. The murine homologue of

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VEGFR-2 (also denoted fetal liver kinase-1; Flk-1), shares 85% sequence identity with human KDR (MATTHEWS et al. 1991). Both VEGFR-1 and VEGFR-2 have seven immunoglobulin (Ig)-like domains in the extracellular domain (ECD), a single transmembrane region and a consensus tyrosine kinase sequence, which is interrupted by a kinase-insert domain (SHIBUYA et al. 1990; TERMAN et al. 1991; MATTHEWS et al. 1991). VEGFR-1 has the highest affinity for rhVEGF$_{165}$, with a K_d of approximately 10–20pM (DE VRIES et al. 1992). VEGFR-2 has a somewhat lower affinity for VEGF: the K_d has been estimated to be approximately 75–125pM (TERMAN et al. 1992).

A cDNA coding an alternatively spliced soluble form of VEGFR-1 (sVEGFR-1), lacking the seventh Ig-like domain, the transmembrane sequence and the cytoplasmic domain, has been identified in HUVEC (KENDALL et al. 1996). This sVEGFR-1 receptor binds VEGF with high affinity (K_d 10–20pM) and is able to inhibit VEGF-induced mitogenesis and may be a physiological negative regulator of VEGF's action (KENDALL et al. 1996).

An additional member of the family of RTKs with seven Ig-like domains in the ECD is VEGFR-3 (also denoted Flt-4; PAJUSOLA et al. 1992; GALLAND et al. 1992; FINNERTY et al. 1993) which, however, is not a receptor for VEGF, but rather binds a newly identified ligand called VEGF-C or VEGF-related peptide (VRP) (see Chapt. 3).

Recent studies have mapped the binding site for VEGF to the second immunoglobulin-like domain of VEGFR-1 and VEGFR-2. Deletion of the second domain of VEGFR-1 completely abolished the binding of VEGF. Introduction of the second domain of VEGFR-2 into an VEGFR-1 mutant lacking the homologous domain restored VEGF binding. However, the ligand specificity was characteristic of the VEGFR-2. To further test this hypothesis, chimeric receptors, where the first three or just the second Ig-like domains of VEGFR-1 replaced the corresponding domains in VEGFR-3, were created. Both swaps conferred upon VEGFR-3 the ability to bind VEGF with an affinity nearly identical to that of wild-type VEGFR-1. Furthermore, transfected cells expressing these chimeric VEGFR-3 receptors exhibited increased DNA synthesis in response to VEGF or PlGF (DAVIS-SMYTH et al. 1996).

An application of these structure–function studies is the generation of inhibitors of VEGF activity. The first three Ig-like domains of VEGFR-1 fused to a heavy chain Fc potently inhibits VEGF bioactivity across species. The Fc may confer sufficient half-life and stability when injected systemically (CHAMOW and ASHKENAZI 1996). Therefore, this agent may be a useful tool in determining the role of endogenous VEGF in several in vivo models.

6.2 Signal Transduction

VEGF has been shown to induce the phosphorylation of at least 11 proteins in bovine aortic endothelial cells (GUO et al. 1995). Phospholipase C (PLC)-γ, and two proteins that associate with PLC-γ were phosphorylated in response to VEGF.

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Furthermore, immunoblot analysis to search for mediators of signal transduction that contain SH2 domains demonstrated that VEGF induces phosphorylation of phosphatidyl inositol 3-kinase, *ras* GTPase activating protein (GAP) and several other proteins. These findings suggest that VEGF promotes the formation of multimeric aggregates of VEGF receptors with proteins that contain SH2 domains. These studies, however, did not identify which VEGF receptor(s) are involved in these events. Recently, it was suggested that NO mediates, at least in part, the mitogenic effect of VEGF on cultured microvascular endothelium isolated from coronary venules (MORBIDELLI et al. 1996). The proliferative effect of VEGF was reduced by pre-treatment of the cells with NO-synthase inhibitors. Exposure of the cells to VEGF induced a significant increment in cyclic guanosine monophosphate (cGMP) levels. These findings suggest that VEGF stimulates proliferation of post-capillary endothelial cells through the production of NO and cGMP accumulation.

Several studies have indicated that VEGFR-1 and VEGFR-2 have different signal transduction properties (WALTENBERGER et al. 1994; SEETHARAM et al. 1995). Porcine aortic endothelial cells lacking endogenous VEGF receptors display chemotaxis and mitogenesis in response to VEGF when transfected with a plasmid coding for VEGFR-2 (WALTENBERGER et al. 1994). In contrast, transfected cells expressing VEGFR-1 lack such responses (WALTENBERGER et al. 1994; SEETHARAM et al. 1995). VEGFR-2 undergoes strong ligand-dependent tyrosine phosphorylation in intact cells, while VEGFR-1 reveals a weak or undetectable response (WALTENBERGER et al. 1994; SEETHARAM et al. 1995). In addition, VEGF stimulation results in weak tyrosine phosphorylation that does not generate any mitogenic signal in transfected NIH 3T3 cells expressing VEGFR-1 (SEETHARAM et al. 1995). These findings agree with other studies showing that PlGF, which binds with high affinity to VEGFR-1, but not to VEGFR-2, lacks direct mitogenic or permeability-enhancing properties or the ability to effectively stimulate tyrosine phosphorylation in endothelial cells (PARK et al. 1994).

It seems, then, that interaction with VEGFR-2 is a critical requirement to induce the full spectrum of VEGF biological responses. In further support of this conclusion, VEGF mutants that bind selectively to VEGFR-2 are fully active endothelial-cell mitogens (KEYT et al. 1996b). These findings led to cast doubt on the role of VEGFR-1 as a truly signaling receptor. However, more recent evidence indicates that VEGFR-1 indeed signals, although our understanding of these events is fragmentary. CUNNINGHAM et al. (1995) have demonstrated an interaction between VEGFR-1 and the p85 subunit of phosphatidyl inositol 3-kinase (CUNNINGHAM et al. 1995), suggesting that p85 couples VEGFR-1 to intracellular signal transduction systems and implicate elevated levels of phosphatidyl inositol (3,4,5) P3 levels in this process (CUNNINGHAM et al. 1995). Also, members of the *Src* family, such as *Fyn* and *Yes*, show an increased level of phosphorylation following VEGF stimulation in transfected cells expressing VEGFR-1, but not VEGFR-2 (WALTENBERGER et al. 1994). Furthermore, it has been shown that a specific biological response, the migration of monocytes in response to VEGF (or PlGF), is mediated by VEGFR-1 (BARLEON et al. 1996).

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6.3 Regulation

The expression of VEGFR-1 and -2 genes is largely restricted to the vascular endothelium. The promoter region of VEGFR-1 has been cloned and characterized and a 1-kb fragment of the 5′ flanking region, essential for endothelial-specific expression, was identified (Morishita et al. 1995). Likewise, a 4-kb 5′ flanking sequence has been identified in the promoter of the human VEGFR-2 that confers endothelial cell-specific activation (Patterson et al. 1995).

Similarly to VEGF, hypoxia has been proposed to play an important role in the regulation of VEGF-receptor gene expression. Exposure of rats to acute or chronic hypoxia led to pronounced upregulation of both VEGFR-1 and VEGFR-2 genes in the lung vasculature (Tuder et al. 1995). Also, VEGFR-1 and -2 mRNAs were substantially upregulated throughout the heart, following myocardial infarction in the rat (Li et al. 1996). However, in vitro studies have yielded unexpected results. Hypoxia increases VEGF receptor number by 50% in cultured bovine retinal capillary endothelial cells, but the expression of VEGFR-2 is not induced although, paradoxically, shows an initial downregulation (Takagi et al. 1996). Brogi et al. (1996) have proposed that the hypoxic upregulation of VEGFR-2 observed in vivo is not direct, but requires the release of an unidentified paracrine mediator from ischemic tissues.

Recent studies have provided evidence of a differential transcriptional regulation of the VEGFR-1 and VEGFR-2 genes by hypoxia (Gerber et al. 1997). When HUVEC were exposed to hypoxic conditions, in vitro, increased levels of VEGFR-1 expression were observed. In contrast, VEGFR-2 mRNA levels were unchanged or slightly repressed. Promoter deletion analysis demonstrated that a 430-bp region of the VEGFR-1 promoter was required for transcriptional activation in response to hypoxia. This region includes a heptamer sequence matching the HIF-1 consensus binding site previously found in other hypoxia inducible genes. The element mediating the hypoxia response was further defined as a 40-bp sequence, including the putative HIF-1 binding site, but was not found in the VEGFR-2 promoter. These findings indicate that, unlike the VEGFR-2 gene, the VEGFR-1 receptor gene is directly upregulated by hypoxia via a hypoxia-inducible enhancer element located at position −976 to −937 of the VEGFR-1 promoter (Gerber et al. 1997). Also, recent studies have shown that both TNF-α (Patterson et al. 1996) and TGF-β (Mandriota et al. 1996) have the ability to inhibit the expression of the VEGFR-2 gene in cultured endothelial cells.

7 Role of VEGF and its Receptors in Physiological Angiogenesis

7.1 Distribution of VEGF, VEGFR-1 and VEGFR-2

The proliferation of blood vessels is crucial for a wide variety of physiological processes, such as embryonic development, normal growth and differentiation, wound healing and reproductive functions.

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During embryonic development, VEGF expression is first detected within the first few days following implantation in the giant cells of the trophoblast (Breier et al. 1992; Jakeman et al. 1993). At later developmental stages in the mouse or rat embryos, the VEGF mRNA is expressed in several organs, including heart, vertebral column, kidney and along the surface of the spinal cord and brain. In the developing mouse brain, the highest levels of mRNA expression are associated with the choroid plexus and the ventricular epithelium (Breier et al. 1992). In the human fetus (16–22 weeks), VEGF mRNA expression is detectable in virtually all tissues and is most abundant in lung, kidney and spleen (Shifren et al. 1994).

In situ hybridization studies have shown that the VEGFR-2 mRNA is expressed in the yolk sac and intraembryonic mesoderm and later on in angioblasts, endocardium and small and large vessel endothelium (Quinn et al. 1993; Millauer et al. 1993). These findings strongly suggested a role for VEGFR-2 in the regulation of vasculogenesis and angiogenesis. Other studies have demonstrated that expression of VEGFR-2 mRNA is first detected in the proximal-lateral embryonic mesoderm, which gives rise to the heart (Yamaguchi et al. 1993). VEGFR-2 is then detectable in endocardial cells of the heart primordia and, subsequently, in the major embryonic and extraembryonic vessels (Yamaguchi et al. 1993). These studies have indicated that VEGFR-2 may be the earliest marker of endothelial-cell precursors. The VEGFR-1 mRNA is selectively expressed in vascular endothelial cells, in both fetal and adult mouse tissues (Peters et al. 1993). Similarly to the high-affinity VEGF binding, the VEGFR-1 mRNA is expressed in both proliferating and quiescent endothelial cells, suggesting a role for VEGFR-1 in the maintenance of endothelial cells (Peters et al. 1993).

VEGF expression is also detectable around microvessels in areas where endothelial cells are normally quiescent, such as kidney glomerulus, pituitary, heart, lung and brain (Ferrara et al. 1992; Monacci et al. 1993). These findings raised the possibility that VEGF may be required not only to induce active vascular proliferation but, at least in some circumstances, also for the maintenance of the differentiated state of blood vessels (Ferrara et al. 1992). In agreement with this hypothesis, Alon et al. (1995) have shown that VEGF acts as a survival factor, at least for the developing retinal vessels. They propose that hyperoxia-induced vascular regression in the retina of neonatal animals is a consequence of inhibition of VEGF production by glial cells. Accordingly, intraocular administration of VEGF to newborn rats at the onset of hyperoxia was able to prevent cell apoptosis and regression of the retinal vasculature (Alon et al. 1995).

7.2 The VEGFR-1, VEGFR-2 and VEGF Gene Knockouts in Mice

Recent studies have demonstrated that both VEGFR-1 and VEGFR-2 are essential for normal development of embryonic vasculature. However, their respective roles in endothelial-cell proliferation and differentiation appear to be distinct (Fong et al. 1995; Shalaby et al. 1995). Mouse embryos homozygous for a targeted mutation in the VEGFR-1 locus died in utero between day 8.5 and day 9.5 (Fong et al.

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1995). Endothelial cells developed in both embryonic and extraembryonic sites, but failed to organize in normal vascular channels. Mice in which the VEGFR-2 gene had been inactivated lacked vasculogenesis and also failed to develop blood islands. Hematopoietic precursors were severely disrupted and organized blood vessels failed to develop throughout the embryo or the yolk sac, resulting in death in utero between day 8.5 and day 9.5 (SHALABY et al. 1995).

However, these findings do not necessarily imply VEGF as being equally essential, since other ligands might potentially activate the VEGFR-1 and -2 and, thus, substitute VEGF's action. Very recent studies (CARMELIET et al. 1996; FERRARA et al. 1996) have generated direct evidence for the role played by VEGF in embryonic vasculogenesis and angiogenesis. Inactivation of the VEGF gene in mice resulted in embryonic lethality in heterozygous embryos, between day 11 and day 12. The VEGF+/ – embryos were growth retarded and also exhibited a number of developmental anomalies. The forebrain region appeared significantly underdeveloped. In the heart region, the outflow region was grossly malformed; the dorsal aortae were rudimentary, and the thickness of the ventricular wall was markedly decreased. The yolk sac revealed a markedly reduced number of nucleated red blood cells within the blood islands. Also, the vitelline veins failed to fuse with the vascular plexus of the yolk sac. Significant defects in the vasculature of other tissues and organs, including placenta and nervous system, were observed. In situ hybridization confirmed expression of VEGF mRNA in heterozygous embryos. Thus, the VEGF+/– phenotype appears to be due to gene dosage and not to maternal imprinting.

While several heterozygous phenotypes have been described (BRANDON et al. 1995), this may be the first example of embryonic lethality following the loss of a single allele of a gene that is not maternally imprinted. Therefore, VEGF and its receptors are essential for blood island formation and angiogenesis, such that even reduced concentrations of VEGF are inadequate to support a normal pattern of development. However, inactivation of the PlGF gene does not result in embryonic lethality, even in the homozygous state (CARMELIET and COLLEN 1997). PlGF–/– mice are viable and fertile, although they may have some impairment of wound healing.

8 Role of VEGF in Corpus Luteum Angiogenesis

The development and endocrine function of the ovarian corpus luteum (CL) are dependent on the growth of new capillary vessels. Although several molecules have been implicated as mediators of CL angiogenesis, at present, there is no direct evidence for the involvement of any. The VEGF mRNA is temporally and spatially related to the proliferation of blood vessels in the rat, mouse and primate ovary and in the rat uterus, suggesting that VEGF is a mediator of the cyclical growth of blood vessels that occurs in the female reproductive tract (PHILLIPS et al. 1990; RAVINDRANATH et al. 1992; SHWEIKI et al. 1993; CULLINAN-BOVE and KOOS 1993).

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Very recently, the hypothesis that VEGF is a mediator of CL angiogenesis has been examined in a rat model of hormonally induced ovulation (Ferrara et al. 1998). Treatment with Flt (1–3)-IgG resulted in virtually complete suppression of CL angiogenesis. This effect was associated with inhibition of CL development and progesterone release. Failure of maturation of the endometrium was also observed. Areas of ischemic necrosis were demonstrated in the CL of treated animals; however, no effect on the pre-existing ovarian vasculature was observed. These findings demonstrate that, in spite of the redundancy of potential mediators, VEGF is essential for CL angiogenesis. Furthermore, they have implications in the control of fertility and the treatment of ovarian disorders characterized by hypervascularity and hyperplasia, such as policystic ovary syndrome.

9 Role of VEGF in Pathological Angiogenesis

9.1 Tumor Angiogenesis

In 1945, Algire and Chalkley, on the basis of microscopic observations of the vascular development of tumor xenografts in transparent chambers in mice, proposed that the growth of solid tumors is dependent on the development of a new vascular supply derived from the host (Algire and Chalkley 1945). In 1971, Folkman proposed inhibition of angiogenesis as a novel strategy to treat cancer (Folkman 1971). Since then, extensive research has been devoted to the identification of tumor angiogenesis factor(s).

Many tumor cell lines secrete VEGF in vitro (Ferrara et al. 1992). In situ hybridization studies have demonstrated that the VEGF mRNA is markedly upregulated in the vast majority of human tumors examined so far. These include: lung (Volm et al. 1997a,b), breast (Brown et al. 1995a; Yoshiji et al. 1996), gastrointestinal tract (Brown et al. 1993b; Suzuki et al. 1996), kidney (Brown et al. 1993a), bladder (Brown et al. 1993a), ovary (Olson et al. 1994), endometrium (Guidi et al. 1996) and uterine cervix (Guidi et al. 1995) carcinomas, angiosarcoma (Hashimoto et al. 1995), germ cell tumors (Viglietto et al. 1996) and several intracranial tumors, including glioblastoma multiforme (Shweiki et al. 1992; Plate et al. 1992; Phillips et al. 1993) and sporadic, as well as VHL syndrome-associated capillary hemangioblastoma (Berkman et al. 1993; Wizigmann Voos et al. 1995). In glioblastoma multiforme and other tumors with significant necrosis, the expression of VEGF mRNA is highest in hypoxic tumor cells adjacent to necrotic areas (Shweiki et al. 1992; Plate et al. 1992; Phillips et al. 1993). A correlation exists between the degree of vascularization of the malignancy and VEGF mRNA expression (Berkman et al. 1993; Wizigmann Voos et al. 1995; Guidi et al. 1995). In virtually all specimens examined, the VEGF mRNA was expressed in tumor cells, but not in endothelial cells. In contrast, the mRNAs for VEGFR-1 and -2 were upregulated in the endothelial cells associated with the tumor (Brown et al.

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1993b; PLATE et al. 1993). These findings are consistent with the hypothesis that VEGF is primarily a paracrine mediator (FERRARA et al. 1993).

Immunohistochemical studies have localized the VEGF protein not only to the tumor cells, but also to the vasculature (PLATE et al. 1992; BROWN et al. 1993b). This localization indicates that tumor-secreted VEGF accumulates in the target cells (QU et al. 1995). Interestingly, recent studies have suggested that the angiogenesis mediated by the human immunodeficiency virus (HIV-1) Tat protein (ALBINI et al. 1996a) requires activation of VEGFR-2 (ALBINI et al. 1996b). Tat induces growth of Kaposi's sarcoma (KS) spindle cells and has been implicated in the vascularity of the KS lesions (ALBINI et al. 1996b).

Elevations in VEGF levels have been detected in the serum of some cancer patients (KONDO et al. 1994). Also, a correlation has been noted between VEGF expression and microvessel density in primary breast cancer sections (TOI et al. 1996). A post-operative survey indicated that the relapse-free survival rate of patients with VEGF-positive tumors was significantly worse than that of VEGF-negative, suggesting that expression of VEGF is associated with stimulation of angiogenesis and with early relapse in primary breast cancer (GASPARINI et al. 1997). A similar correlation has been described in gastric-carcinoma patients (MAEDA et al. 1996). VEGF-positivity in tumor sections was correlated with vessel involvement, lymph node metastasis and liver metastasis. Furthermore, patients with VEGF-positive tumors had a worse prognosis than those with VEGF-negative tumors (MAEDA et al. 1996).

The availability of specific monoclonal antibodies capable of inhibiting VEGF-induced angiogenesis in vivo and in vitro (KIM et al. 1992) made it possible to generate direct evidence for a role of VEGF in tumorigenesis. In a study published by KIM et al. (1993), such antibodies were found to exert a potent inhibitory effect on the growth of three human tumor cell lines injected subcutaneously in nude mice, the SK-LMS-1 leiomyosarcoma, the G55 glioblastoma multiforme and the A673 rhabdomyosarcoma. The growth inhibition ranged between 70% and more than 95%. Subsequently, other tumor cell lines were found to be inhibited in vivo by this treatment (WARREN et al. 1995; MELNYK et al. 1996; ASANO et al. 1995; BORGSTROM et al. 1998a).

In agreement with the hypothesis that inhibition of neovascularization is the mechanism of tumor suppression, the density of blood vessels was significantly lower in sections of tumors from antibody-treated animals than in controls. Furthermore, neither the antibodies nor VEGF had any effect on the in vitro growth of the tumor cells (KIM et al. 1993). Intravital videomicroscopy techniques have allowed a more direct verification of the hypothesis that anti-VEGF antibodies indeed block tumor angiogenesis (BORGSTROM et al. 1996). Non-invasive imaging of the vasculature revealed a nearly complete suppression of tumor angiogenesis in anti-VEGF treated animals compared with controls, at all time points examined (BORGSTROM et al. 1996).

VEGF is a mediator of the in vivo growth of human colon carcinoma HM7 cells in a nude mouse model of liver metastasis (WARREN et al. 1995). Treatment with anti-VEGF monoclonal antibodies resulted in a dramatic decrease in the

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number and size of metastases. Similarly, administration of anti-VEGF neutralizing antibodies inhibited primary tumor growth and metastasis of A431 human epidermoid carcinoma cells in severe combined immune deficient (SCID) mice (MELNYK et al. 1996) or HT-1080 fibrosarcoma cells implanted in BALB/c nude mice (ASANO et al. 1995).

Recently, BORGSTROM et al. (1998b) have shown that a combination treatment that includes anti-VEGF monoclonal antibody and doxorubicin results in a significant enhancement of the efficacy of either agent alone and led, in some cases, to complete regression of tumors derived from MCF-7 breast carcinoma cells in nude mice.

Intravital fluorescence microscopy and video imaging analysis have also been applied to address the important issue regarding the effects of VEGF on permeability and other properties of tumor vessels (YUAN et al. 1996). Treatment with anti-VEGF monoclonal antibodies was initiated after tumor xenografts had already been established and vascularized, and resulted in time-dependent reductions in vascular permeability (YUAN et al. 1996). These effects were accompanied by striking changes in the morphology of vessels, with dramatic reduction in diameter and tortuosity. This reduction in diameter is expected to block the passage of blood elements and eventually stop the flow in the tumor vascular network. A regression of blood vessels was observed after repeated administrations of anti-VEGF antibody. These findings suggest that tumor vessels require constant stimulation with VEGF in order to maintain not only their proliferative properties, but also some key morphological features (YUAN et al. 1996).

An independent verification of the hypothesis that the VEGF action is required for tumor angiogenesis has been provided by the finding that retrovirus-mediated expression of a dominant negative VEGFR-2 mutant, which inhibits signal transduction through wild-type VEGFR-2, suppresses the growth of glioblastoma multiforme and other tumor cell lines in vivo (MILLAUER et al. 1994).

9.2 Angiogenesis Associated with Other Pathological Conditions

Diabetes mellitus, occlusion of central retinal vein or prematurity with subsequent exposure to oxygen can all be associated with intraocular neovascularization (GARNER 1994). The new blood vessels may lead to vitreous hemorrhage, retinal detachment, neovascular glaucoma and eventual blindness (GARNER 1994). Diabetic retinopathy is the leading cause of blindness in the working population (OLK and LEE 1993). All of these conditions are known to be associated with retinal ischemia (PATZ 1980). In 1948, MICHAELSON proposed that a key event in the pathogenesis of these conditions was the release by the ischemic retina of diffusible angiogenic factor(s) ("factor X") responsible for retinal and iris neovascularization into the vitreous (MICHAELSON 1948). VEGF, by virtue of its diffusible nature and hypoxia-inducibility, was an attractive candidate as a mediator of intraocular neovascularization. Accordingly, elevations of VEGF levels in the aqueous and

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vitreous of eyes with proliferative retinopathy have been described (AIELLO et al. 1994; ADAMIS et al. 1994; MALECAZE et al. 1994).

In a large series, a strong correlation was found between levels of immunoreactive VEGF in the aqueous and vitreous humors and active proliferative retinopathy. VEGF levels were undetectable or very low (< 0.5ng/ml) in the eyes of patients affected by non-neovascular disorders or diabetes without proliferative retinopathy (AIELLO et al. 1994). In contrast, the VEGF levels were in the range 3–10ng/ml in the presence of active proliferative retinopathy associated with diabetes, occlusion of central retinal vein or prematurity. In agreement with these findings, in situ hybridization studies have demonstrated upregulation of VEGF mRNA in the retina of patients with proliferative retinopathies secondary to diabetes, central retinal vein occlusion, retinal detachment or intraocular tumors (PE'ER et al. 1996).

More direct evidence for a role of VEGF as a mediator of intraocular neovascularization has been generated in a primate model of iris neovascularization and in a murine model of retinopathy of prematurity (MILLER et al. 1994; PIERCE et al. 1995). In the former, intraocular administration of anti-VEGF antibodies dramatically inhibits the neovascularization that follows occlusion of central retinal veins (ADAMIS et al. 1996). Likewise, soluble VEGFR-1 or VEGFR-2 fused to an IgG suppresses retinal angiogenesis in the mouse model (AIELLO et al. 1995).

Neovascularization is also a major cause of visual loss in age-related macular degeneration (AMD), the overall leading cause of blindness (GARNER 1994). Most AMD patients demonstrate atrophy of the retinal pigment epithelia and characteristic formations called "drusen". A significant percentage of AMD patients (~20%) manifest the neovascular (exudative) form of the disease. In this condition, the new vessels stem from the extraretinal choriocapillary (GARNER 1994). Leakage and bleeding from these vessels may lead to damage to the macula and, ultimately, to loss of central vision. Because of the proximity of the lesions to the macula, laser photocoagulation or surgical therapy are of very limited value. Very recent studies have documented the immunohistochemical localization of VEGF in surgically resected choroidal neovascular membranes from AMD patients (LOPEZ et al. 1996; KVANTA et al. 1996). These findings suggests a role for VEGF in the progression of AMD-related choroidal neovascularization, raising the possibility that a pharmacological treatment with monoclonal antibodies or other VEGF inhibitors may constitute a therapy for this condition.

Two independent studies have suggested that VEGF is involved in the pathogenesis of rheumatoid arthritis (RA), an inflammatory disease in which angiogenesis plays a significant role (KOCH et al. 1994; FAVA et al. 1994). The RA synovium is characterized by the formation of pannus, an extensively vascularized tissue, which invades and destroys the articular cartilage (FASSBENDER and SIMLING-ANNENFELD 1983). Levels of immunoreactive VEGF were found to be high in the synovial fluid of RA patients, while they were very low or undetectable in the synovial fluid of patients affected by other forms of arthritis or by degenerative joint disease (KOCH et al. 1994; FAVA et al. 1994). Furthermore, anti-VEGF antibodies significantly reduced the endothelial cell chemotactic activity of the RA synovial fluid (KOCH et al. 1994).

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It has been shown that VEGF expression is increased in psoriatic skin (DETMAR et al. 1994). Increased vascularity and permeability are characteristic of psoriasis. Also, VEGF mRNA expression has been examined in three bullous disorders with subepidermal blister formation, bullous pemphigoid, erythema multiforme and dermatitis herpetiformis (BROWN et al. 1995b).

Angiogenesis is also important in the pathogenesis of endometriosis, a condition characterized by ectopic endometrium implants in the peritoneal cavity. Recently, elevation of VEGF in the peritoneal fluid of patients with endometriosis has been reported (MCLAREN et al. 1996; SHIFREN et al. 1996). Immunohistochemistry indicated that activated peritoneal fluid macrophages, as well as tissue macrophages within the ectopic endometrium, are the main source of VEGF in this condition. (MCLAREN et al. 1996; SHIFREN et al. 1996). VEGF upregulation has also been implicated in the hypervascularity of the ovarian stroma that characterizes Stein-Leventhal syndrome (KAMAT et al. 1995). Moreover, SATO et al. (1995) proposed that VEGF may be responsible for the characteristic hypervascularity of Graves' disease. Thyroid-stimulating hormone (TSH), insulin phorbol ester, dibutiryl cAMP and Graves' IgG were found to stimulate VEGF mRNA expression in cultured human thyroid follicles (SATO et al. 1995).

10 VEGF and Therapeutic Angiogenesis

The availability of agents able to promote the growth of new collateral vessels would be, potentially, of major therapeutic value for disorders characterized by inadequate tissue perfusion, and might constitute an alternative to surgical reconstruction procedures. For example, chronic limb ischemia, most frequently caused by obstructive atherosclerosis affecting the superficial femoral artery, is associated with a high rate of morbidity and mortality, and treatment is currently limited to surgical revascularization or endovascular interventional therapy (GRAOR and GRAY 1991); no pharmacological therapy has been shown to be effective for this condition.

It has been shown that intraarterial or intramuscular administration of recombinant human (rh)$VEGF_{165}$ may significantly augment perfusion and development of collateral vessels in a rabbit model, in which chronic hindlimb ischemia was created by surgical removal of the femoral artery (TAKESHITA et al. 1994). These studies provided angiographic evidence of neovascularization in the ischemic limbs. Arterial gene transfer with cDNA encoding VEGF also led to revascularization in the same rabbit model to an extent comparable with that achieved with the recombinant protein (TAKESHITA et al. 1996a,b). In addition, the hypothesis that the angiogenesis initiated by the administration of VEGF improved muscle function in ischemic limbs was tested by WALDER et al. (1996). A single intraarterial injection of $rhVEGF_{165}$ augmented muscle function in this rabbit model of peripheral limb ischemia. This exercise-induced hyperemia was signifi-

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cantly improved in ischemic limbs treated with rhVEGF$_{165}$ (WALDER et al. 1996). Such improvement in perfusion was, however, not seen in other non-ischemic tissues, including the contralateral limb. Similarly, BAUTERS et al. (1994) have shown that both maximal flow velocity and maximal blood flow are significantly increased in ischemic limbs following VEGF administration.

Other studies have shown that VEGF administration also leads to a recovery of normal endothelial reactivity in dysfunctional endothelium. Following obstruction of a large artery and development of collateral vessels, the increase in blood flow that normally follows acetylcholine infusion is severely blunted; serotonin paradoxically leads to a decrease in blood flow (BAUTERS et al. 1995). Thirty days after a single intraarterial bolus of VEGF$_{165}$, restoration of the normal increase in blood flow was demonstrated in the ischemic rabbit hindlimb, following acethylcholine or serotonin infusion (BAUTERS et al. 1995).

BANAI et al. (1994a) have shown that VEGF administration results in increased coronary blood flow in a dog model of coronary insufficiency. Following occlusion of the left circumflex coronary artery, daily intraluminal injections of rhVEGF distal to the occlusion resulted in a significant enhancement of collateral blood flow over a 4-week period. In addition, HARADA et al. (1996) demonstrated that extraluminal administration of as little as 2μg of rhVEGF by an osmotic pump results in a significant increase in coronary blood flow in a pig model of chronic myocardial ischemia created by ameroid occlusion of the left proximal circumflex artery. Also, magnetic resonance imaging provided a non-invasive assessment of the benefits secondary to VEGF administration in the porcine model (PEARLMAN et al. 1995). Image series converted to a space–time map demonstrated a reduction in the size of the ischemic zone and a decreased delay in contrast arrival after VEGF treatment. These findings demonstrated improvement in cardiac global and regional function and reduced infarct size, resulting from enhanced collateral blood supply (PEARLMAN et al. 1995; WARE and SIMONS 1997).

A further potential therapeutic application of VEGF is the prevention of restenosis following percutaneous transluminal angioplasty (PTA). Between 15% and 75% of patients undergoing PTA for occlusive coronary or peripheral arterial disease develop restenosis within 6 months (GRAOR and GRAY 1991). It has been proposed that damage to the endothelium is a crucial event, triggering fibrocellular intimal proliferation (ESSED et al. 1983). Therefore, the induction of rapid re-endothelialization may be an effective strategy to prevent the cascade of events leading to neointima formation and ultimately to restenosis in patients. Recent evidence shows that VEGF accelerates re-endothelialization and also attenuates intimal hyperplasia in balloon-injured rat carotid artery or rabbit aorta (ASAHARA et al. 1995; CALLOW et al. 1994).

Recently, the hypothesis that VEGF may result in therapeutically significant angiogenesis in humans has been tested by ISNER et al. (1996b) in a gene-therapy trial in patients with severe limb ischemia. A case report of an interim analysis of this trial has been published (ISNER et al. 1996a). Arterial gene transfer of 2000μg naked plasmid DNA encoding VEGF$_{165}$, applied to the hydrogel polymer coating of an angioplasty balloon, resulted in angiographic and histologic evidence of

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angiogenesis in the knee mid-tibial and ankle levels 4 weeks after transfer. Such effects persisted at a 12-week view (ISNER et al. 1996a).

11 Conclusions

The recent findings that heterozygous mutations inactivating the VEGF gene result in profound deficits in vasculogenesis and blood island formation, leading to early intrauterine death, emphasize the pivotal role played by this molecule in the development of the vascular system. Future studies, using inducible gene knockout technology (KUHN et al. 1995) should help determine the timing, when the embryo is most vulnerable to VEGF deficiency.

The elucidation of the signal transduction properties of the VEGF receptors holds the promise to dissect the pathways leading to such fundamental biological events as endothelial cell differentiation, morphogenesis and angiogenesis. Furthermore, a more complete understanding of the signaling events involving other endothelial cell-specific tyrosine kinases as well as cell-adhesion molecules and their interrelation with the VEGF/VEGF receptor system should provide a more integrated view of the biology of the endothelial cell, both in normal and abnormal circumstances. In this context, recent studies have shown that VEGF-mediated angiogenesis requires a specific vascular integrin pathway, mediated by $\alpha v\beta 5$ (FRIEDLANDER et al. 1995). Furthermore, a ligand selective for the endothelial cell-specific tyrosine kinase Tie-2 has been recently identified and named angiopoietin (Ang)-1 (DAVIS et al. 1996). Gene knockout studies have shown that Ang-1 is required for the correct assembly of the vessel wall (SURI et al. 1996). Ang-1 seems to play a crucial role in mediating reciprocal interactions between the endothelium and surrounding matrix and mesenchyme, and plays a later role in angiogenesis than VEGF. Also, unlike VEGF, Ang-1 does not directly stimulate endothelial cell growth. Interestingly, very recent studies provide evidence for the existence of Ang-2, a natural antagonist for the Tie-2 receptor. (MAISONPIERRE et al. 1997). Transgenic expression of Ang-2 disrupted blood vessel formation. The interrelation between the VEGF and Ang systems is likely to be an area of intense investigation in vascular biology.

An attractive possibility is that recombinant VEGF or gene therapy with the VEGF gene may be used to promote endothelial cell growth and collateral vessel formation. This would represent a novel therapeutic modality for conditions that frequently are refractory to conservative measures and unresponsive to pharmacological therapy. $rhVEGF_{165}$ is already in clinical trials for the treatment of myocardial ischemia associated with coronary artery disease.

The high expression of VEGF mRNA in human tumors, the presence of the VEGF protein in ocular fluids of individuals with proliferative retinopathies and in the synovial fluid of RA patients, as well as the localization of VEGF in AMD lesions, strongly supports the hypothesis that VEGF is a key mediator of angio-

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genesis associated with various disorders. Therefore, anti-VEGF antibodies or other inhibitors of VEGF, used alone or in combination with other agents, may be of therapeutic value for a variety of malignancies and other disorders. Very recently, a humanized version of a high-affinity anti-VEGF monoclonal antibody, which retains the same affinity and efficacy as the original murine antibody, has been generated (PRESTA et al. 1997) and is being tested in humans as a treatment for solid tumors, alone or in combination with chemotherapy.

In conclusion, in spite of the plurality of factors potentially involved in angiogenesis, one specific factor, VEGF, appears to play an irreplaceable role in a variety of physiological and pathological circumstances.

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Promoting the formation of new collateral vessels in ischemic tissues using angiogenic growth factors (therapeutic angiogenesis) is a an exciting frontier of cardiovascular medicine. Conversely, inhibition of the action of key regulators of angiogenesis, such as VEGF, constitutes a promising approach for the treatment of solid tumors and intraocular neovascular syndromes. These concepts are being tested now in clinical trials.

Clinical applications of angiogenic growth factors and their inhibitors

NAPOLEONE FERRARA[1] & KARI ALITALO[2]

In embryos, blood vessels form through two distinct processes, vasculogenesis and angiogenesis. Vasculogenesis involves the de novo differentiation of endothelial cells from mesodermal precursors, whereas in angiogenesis new vessels are generated from pre-existing ones[1]. Vasculogenesis takes place only during embryonic development and leads to the formation of a primary vascular plexus. Later these rather uniformly sized endothelial channels are remodeled into a mature system consisting of a tree-like hierarchy of large and small vessels. New capillaries then form through angiogenesis, either by sprouting or by splitting (intussusception) from their vessels of origin. In adults, angiogenesis is essential for the female reproductive cycle, and for repair, remodeling and regeneration of tissues, for example during wound healing[2]. Neovascularization is also important in pathological processes such as tumor growth and metastasis[2].

The known endothelial cell specific growth factors and their receptors can be classified into vascular endothelial growth factor (VEGF) and angiopoietin (Ang) families[3] (Fig. 1).Among the various angiogenic factors, VEGF is probably the most essential for the development and differentiation of the vascular system[4]. Loss of a single VEGF allele results in embryonic lethality[5,6] (Fig. 2) . Even selective inactivation of the heparin-binding isoforms of VEGF, leaving one functional isoform ($VEGF_{120}$), is insufficient for the proper development of the cardiovascular system and results in myocardial ischemia and perinatal or early postnatal lethality[7]. Also, other angiogenic factors, such as FGFs may work more indirectly, some of them through the VEGFs and their receptors[8], so that a thorough knowledge of the signal transduction pathways of VEGFs and angiopoietins is essential for their use in therapeutic settings.

Therapeutic angiogenesis and inhibition of arterial restenosis

An exciting frontier of cardiovascular medicine is therapeutic angiogenesis. Promoting the formation of new collateral vessels on the ischemic myocardium, leg muscles and other tissues would have an important effect on the treatment of disorders for which pharmacological intervention has been ineffective in controlled trials and for which therapy is now limited to surgical revascularization or endovascular interventional therapy[9] .

Several angiogenic molecules have been tested in animal models, including bFGF, aFGF, FGF-5, VEGF isoforms, VEGF-C, HGF/SF and Ang-1/Ang-2. The factors tested most extensively are VEGF and bFGF. In some cases, the recombinant protein was tested. In others, gene transfer using naked DNA or adenoviral vectors was used. A single intra-arterial administration of 500–1000 μg of $rhVEGF_{165}$ augmented perfusion and development of collateral vessels in a rabbit model of hindlimb ischemia in which the femoral artery was surgically removed[10]. Similar results were obtained in the same model with intramuscular or intra-arterial administration of aFGF, bFGF, HGF/SF and VEGF-C (refs. 11–14). VEGF administration after removal of the femoral artery not only resulted in increased vascularization but also led to recovery of the normal endothelial reactivity to various mediators[15]. Arterial gene transfer with cDNA encoding VEGF isoforms also led to revascularization to an extent comparable to that achieved with the recombinant protein[16]. Moreover, administration of a $VEGF_{165}$ adenovirus vector shortly after common iliac artery ligation in the rat was capable of stimulating an angiogenic response that protects against subsequent occlusion of the femoral artery, indicating that gene transfer of VEGF might be useful in the prophylaxis of advancing arterial occlusive disease[17]. As little as 2 μg rhVEGF delivered over 4 weeks periadventitially, distal to the occlusion, resulted in a significant increase in coronary blood flow and functional improvement in a pig model of chronic myocardial ischemia[18]. Very similar results were obtained using bFGF (ref. 19). Unexpectedly, even a single intracoronary administration of VEGF (or bFGF) was efficacious in this model to an extent comparable to that of 4-week infusion, despite the fact that only a small fraction of protein localizes to the ischemic area[20]. Given such results, it is conceivable that young and otherwise healthy animals are very responsive to exogenous growth factors in the context of ischemia. At least some of this responsiveness may be due to the upregulation of VEGF receptors in the endothelia of ischemic tissues[21]. Adenovirus-mediated gene transfer of $VEGF_{121}$ (ref. 22) or FGF-5 (ref. 23) also resulted in collateral vessel growth and functional improvement in porcine models of myocardial ischemia.

These encouraging animal studies led to clinical trials using recombinant $VEGF_{165}$, aFGF, bFGF or gene therapy with plasmid or with adenoviral vectors. There is considerable debate whether gene therapy or administration of recombinant protein would be preferable. Delivery of angiogenic proteins by gene therapy might not only minimize their systemic side effects, such as hypotension (VEGF) or nephrotoxicity (bFGF), but also provide a slow release of the encoded factor for 1–2 weeks, leading to a more lasting angiogenic response. However, slow release of the recombinant protein, using microspheres or heparin-alginate formulations, might achieve the same results, without the potential risks associated with the use of viral vectors.

Arterial gene transfer of naked plasmid DNA encoding $VEGF_{165}$ in a patient with severe limb ischemia produced angiographic and histologic evidence of angiogenesis in the knee, mid-tibial and ankle levels 4 weeks after the transfer[24]. In a subsequent study, the $VEGF_{165}$ plasmid cDNA was injected intramuscularly[25]. Gene transfer was done in ten limbs of nine patients with nonhealing ischemic ulcers and/or rest pain due

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to peripheral arterial disease. Improvement in the ankle-brachial index and distal flow in eight limbs were reported[25]. Additional small trials by the same group have also shown that local injection of the $VEGF_{165}$ plasmid DNA resulted in clinical improvement in patients affected by myocardial ischemia[26] or Burger's disease (thromboangiitis obliterans)[27]. However, none of these studies were placebo-controlled. Clinical trials using VEGF-C naked DNA or adenovirus mediated gene transfer of $VEGF_{121}$ in myocardial ischemia patients are now in phase I. Femoral angiograms from a patient with limb ischemia, before and 3 months after transfection of a $VEGF_{165}$ plasmid/liposome expression vector, show increased vascular density after the treatment (Fig. 3). However, the trial is ongoing and some caution should be used in interpreting such data, until more patients and the effect of placebo are more extensively evaluated.

Clinical trials using recombinant $VEGF_{165}$ and bFGF are also ongoing. In a phase I study in patients with coronary ischemia in which $rhVEGF_{165}$ was administered by intracoronary infusion, the molecule was safely tolerated at all doses tested[28]. There was evidence of improvement in perfusion in seven of fifteen subjects and improved collateralization in five of seven who underwent follow-up coronary angiography. However, a subsequent placebo-controlled phase II study, in which rhVEGF was delivered as a single intracoronary infusion, followed by three intravenous infusions, has not demonstrated clinical benefit[29]. The treatment was not better than placebo in treadmill time and pain relief, at least at 60 days[29]. Brief exposures to $rhVEGF_{165}$, such as those achieved in this trial, may be insufficient to trigger and maintain a therapeutically meaningful angiogenic response, especially in the context of extensive atherosclerotic disease. Also, systemic administration of $rhVEGF_{165}$ or other factor may fail to generate an appropriate angiogenic concentration gradient from ischemic to non-ischemic areas, a requisite aspect of angiogenesis in a variety of physiological and pathological circumstances[1]. Moreover, the placebo effect is probably greater than initially suspected, and even patients with very compromised myocardial function may show a substantial improvement with placebo. A phase II study with bFGF for coronary ischemia is now ongoing.

Local gene transfer into the vascular wall offers a promising alternative for the treatment of the complication of restenosis after percutaneous transcoronary angioplasty (PTCA) and coronary stenting. Restenosis occurs in many treated patients in 6 months, leading to obstruction in 20–35% of the patients[30]. The pathogenesis of restenosis depends on endothelial damage, which also predisposes arteries to other pathological conditions, such as spasms or thrombosis. Prophylaxis of restenosis could therefore be based on strategies for endothelial protection or enhancement of endothelial repair and endothelial growth factors or vascular gene transfer could be used for this[31]. Re-endothelization in balloon-injured rat carotid artery was accelerated by a single dose of recombinant VEGF injected into the bloodstream or locally[32,33]. Vessel status was also improved by injection of VEGF plasmid into adventitial surface of rabbit carotid arteries[34]. Intravascular gene transfer in the arterial wall was not very efficient[35], but secreted proteins such as VEGF could be used for therapeutic gene transfer trials using infusion-perfusion catheters[36] or histamine-induced increase of endothelial permeability[37]. Because VEGF and VEGF-C share one receptor (VEGFR-2) but differ in the other receptor, VEGF-C and $VEGF_{165}$ might have overlapping but distinct effects in the vessel wall. However, VEGF-C gene transfer inhibits intimal thickening early, and the protective effect is at least equal to that seen with $VEGF_{165}$ gene transfer[38].

Therapeutic inhibition of vascular endothelial growth factor

Tumors

The growth of tumor xenografts in transparent chambers in mice is preceded by an increase in vascular density, indicating that the rapid growth of tumors depends on the development of a neovascular supply[39]. In 1971, inhibition of angiogenesis was proposed as a valid strategy for the treatment of solid tumors and the search for the mediator(s) of tumor angiogenesis was begun[40].

Although inhibition of bFGF (ref. 41) or angiopoietin/Tie2 (refs. 42,43) may inhibit tumor growth, so far VEGF and its receptors constitute the most extensively investigated system in tumor angiogenesis and are now a main target of anti-cancer strategies. VEGF mRNA is substantially upregulated in most human tumors[4]. Although tumor cells represent the main source of VEGF, tumor-associated stroma is also an important site of VEGF production[44]. There is a correlation between VEGF expression and microvessel density in primary breast cancer sections[45]. A similar correlation has been described in several other malignancies, including gastric carcinoma[46]. Furthermore, there are increases in plasma levels of VEGF in tumor patients compared with tumor-free individuals, and high VEGF levels before chemotherapy are associated with a poor outcome[47].

Direct evidence for involvement of VEGF in tumorigenesis was first demonstrated using monoclonal antibodies against

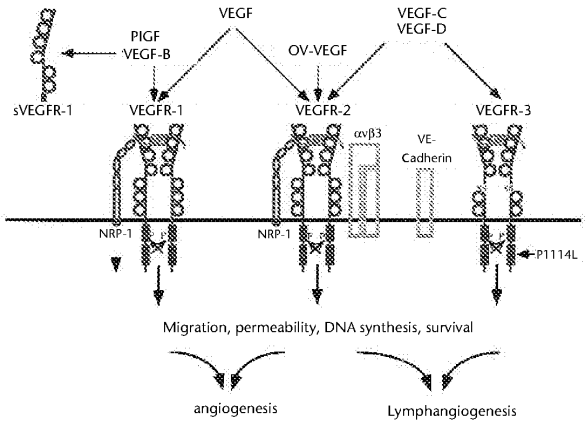


Fig. 1 VEGFs, their receptors and some of their endothelial effects in cells and tissues. Ligand binding induces receptor dimerization and subsequent auto/transphosphorylation, activates various signal transduction pathways and leads to differential cellular responses. sVEGFR-1, soluble VEGFR-1; HSPG, heparan sulphate proteoglycan; NP-1, neuropilin-1; a_vb_3, integrin a_vb_3 (reported to make a molecular complex with activated VEGFR-2; ref. 95). VE-cadherin is also able to form a complex with VEGFR-2, a requirement for VEGF-dependent anti-apoptotic signals involving the PI3-kinase/Akt pathway[96]. P1114L, point mutation of VEGFR-3 affecting patients in a family with lymphoedema[97].

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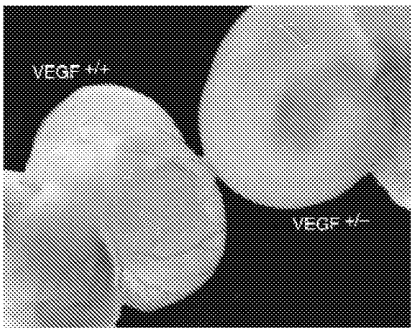


Fig. 2 Yolk sac of E10.5 VEGF$^{+/+}$ and VEGF $^{+/-}$ mouse embryos[5]. There is an apparent absence of vasculature in the yolk sac of the heterozygous, which die around E11. This is probably the only example among vertebrates of lethality after inactivation of a single allele of a gene that is not maternally imprinted.

VEGF in human xenografts in nude mice[48]. These initial studies showed that several tumor cell lines can be substantially growth-inhibited by this treatment[48]. These findings were extended to a broad variety of tumor cell lines, including carcinomas, sarcomas and gliomas[4]. Intravital videomicroscopy techniques have augmented our understanding of VEGF in tumorigenesis[49,50]. Non-invasive imaging of the vasculature demonstrated a nearly complete suppression of tumor-associated angiogenesis in animals treated with monoclonal antibodies against VEGF compared with controls, providing a direct verification that inhibition of angiogenesis is the mechanism of tumor suppression after anti-VEGF treatment[49]. Intravital microscopy techniques have also been used to investigate the effects of VEGF on the permeability and other properties of tumor vessels[50]. Treatment with antibodies against VEGF resulted in time-dependent reductions in vascular permeability, in the diameter and tortuosity and eventually to a regression of tumor blood vessels; thus, VEGF is also an essential survival factor for tumor endothelial cells[50]. Further evidence that VEGF action is required for tumor angiogenesis has been provided by the finding that retrovirus-mediated expression of a dominant negative VEGFR-2 mutant, which inhibits signal transduction through wild-type VEGFR-2 receptor, suppresses the growth of glioblastoma multiforme as well as other tumor cell lines *in vivo*[51]. Furthermore, high local expression of the soluble extracellular domain of VEGFR-1 or VEGFR-2, achieved by administration of the recombinant proteins, adenoviral-mediated gene transfer or by stable transfection of tumor cells, may significantly inhibit tumor growth, metastasis and mortality rate in nude mice[52,53].

Several strategies have been used to generate VEGF inhibitors suitable for clinical trials. One approach involves the 'humanization' of mouse monoclonal antibodies. A chief advantage of 'humanized' antibodies is a high degree of specificity, combined with a long half-life and little or no immunogenicity. A 'humanized' high-affinity monoclonal antibody against VEGF (rhuMAb VEGF) with the same affinity and biological properties as the original murine antibody has been described[54]. Toxicological studies in primates have shown that the effects of rhuMAb VEGF are limited to inhibition of angiogenesis in the female reproductive tract and in the epiphyseal growth plate in sexually immature animals that have not completed statural growth[55]. rhuMAb VEGF is now in phase II clinical trials for the treatment of non-small cell lung carcinoma and colorectal carcinoma in conjunction with standard chemotherapy and for breast and renal cell carcinoma as a single agent. In addition, small molecules that inhibit VEGFR-2 signal transduction are undergoing phase II clinical trials in cancer patients[56]. Furthermore, monoclonal antibodies against VEGFR-2 are entering clinical trials.

Retinal ischemia and other conditions

Diabetes mellitus, occlusion of the central retinal vein or prematurity with subsequent exposure to oxygen can all be associated with intraocular neovascularization[57]. A common denominator among these conditions is retinal ischemia[57]. The new blood vessels may lead to vitreous hemorrhage, retinal detachment, neovascular glaucoma, and eventual blindness. Diabetic retinopathy is the leading cause of blindness in the working population. The hypothesis that ischemia-induced VEGF may be pathogenic in these conditions was initially tested by measuring VEGF levels in the eye fluids of patients. In a large series with 165 patients, a strong correlation was found between concentrations of VEGF in both aqueous and vitreous and active proliferative retinopathy associated with diabetes, occlusion of central retinal vein or prematurity[58]. Direct evidence for the role of VEGF as a mediator of intraocular neovascularization has been generated in several animal models, including a primate model of iris neovascularization and a mouse model of retinopathy of prematurity. In the former, intraocular administration of monoclonal antibodies against VEGF substantially inhibits the neovascularization that follows the occlusion of central retinal veins[59]. Likewise, soluble VEGFR-1 or VEGFR-2 extracellular domains fused to the immunoglobulin γ Fc domain suppress retinal angiogenesis in the mouse model[60]. There is also evidence that growth hormone/insulin-like growth factor-1 is involved in ischemia-induced retinal neovascularization[61].

Neovascularization is a principal cause of visual loss also in the wet form of age-related macular degeneration (AMD), the overall leading cause of blindness[62]. Several studies have documented the immunohistochemical localization of VEGF in surgically resected choroidal neovascular membranes from AMD patients[63]. These findings suggest involvement of VEGF in the progression of AMD-related choroidal neovascularization. Anti-VEGF strategies for AMD are now being explored in clinical trials. One approach consists in the intravitreal administration of a recombinant humanized anti-VEGF Fab antibody fragment. Another strategy involves the injection of 2′-fluoropyrimidine RNA oligonucleotide ligands (aptamers)[64].

VEGF inhibition may also have therapeutic value for the treatment of ischemic-reperfusion related brain edema and injury. VEGF antagonism has shown beneficial effects in a mouse model of cortical ischemia[65]; reducing acutely the volume of edematous tissue and resulting in a significant sparing of cortical tissue.

VEGF is important in angiogenesis in the female reproductive tract. VEGF inhibition results in suppression of corpus luteum angiogenesis in rodents[66] and primates[55]. VEGF inhibitors might be used to treat conditions characterized by ovarian hyperplasia and hypervascularity, such as the polycystic ovary syndrome[66]. VEGF-dependent angiogenesis may also be important pathogenically in endometriosis. Furthermore, VEGF is a

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mediator of the ovarian growth and increased vascular permeability of ovarian hyperstimulation syndrome, a potentially fatal condition characterized by massive ovarian enlargement that may follow medical induction of ovulation with gonadotropins[67].

Perspectives

$VEGF_{165}$ binds to neuropilin-1, which functions as a ligand binding subunit of putative transmembrane receptors mediating specific signals for different semaphorins, the molecules mediating the collapse of axonal growth cones[68]. Neuropilin is expressed in endothelial cells and enhances the mitogenic effects of VEGFR-2 upon $VEGF_{165}$ stimulation. Thus, there may be an as-yet ill-defined cross-regulation of cellular signals between these two families of factors. These findings lead to the intriguing conclusion that the processes of axon guidance and development of a network of capillary tubes share at least some common molecular mechanisms. In addition, the angiopoietin receptor/Tie and ephrin families of endothelial tyrosine kinases have important functions in the formation and maintenance of the vascular system[69-71]. Endothelial cell-specific members of the TGF-β receptor and Notch families have also been described[72,73]. Given this complexity of vascular endothelial signaling, therapies using VEGF alone or any other single angiogenic factor may produce incompletely functioning or unstable endothelial channels with defective arteriovenous and pericellular differentiation, characteristic of many tumors[74]. Combinations of growth factors may be preferable in future therapies directed to neovascularization of tissues, with an adequate investment of the formed vessels with periendothelial matrix and pericyte/smooth muscle cells. In fact, a more heterogenous set of genes coordinating angiogenic functions may be provided by active ongoing research of hypoxia-regulated gene expression in mammalian cells[75]. Also, some virus-encoded proteins, such as the VEGFR-2 activating HIV Tat protein[76], Kaposi sarcoma herpesvirus-associated G-protein-coupled receptor[77] or Orf virus encoded VEGF-E[78-80] may offer new insights into the mechanism of regulation of angiogenesis.

Although recent research has focused on the combination of VEGF and Ang-1 as being especially promising, it is not known now which growth factor combinations will prove to be the most effective therapeutically. VEGF and bFGF have a very synergistic effect in the induction of angiogenesis, both *in vitro* and *in vivo*[4]. The interaction between VEGF and HGF/SF is also being actively investigated. Although transgenic expression of Ang-1 in the skin epidermis under the keratin (K)14 promoter has been associated with neovascularization[81], other studies, using defined amounts of the recombinant protein in a model of adult neovascularization, have failed to demonstrate strong angiogenic responses to Ang-1, unless it is used in combination with VEGF (refs. 71,82). This discrepancy may be explained by the fact that the expression of the K14 promoter is initiated already at midgestation, and thus the results may reflect persistence of the fetal neovascularization. It is possible, however, that Ang-1 may provide a co-factor for combination therapies. A further unresolved issue is the correct dosage of growth factor(s). This seems particularly important for a molecule like VEGF, which has several isoforms and such a tight dose–response effect that a 50% reduction in expression results in lethality during embryonic life[5,6]. Conversely, continuous local overexpression of VEGF may result in a hemangioma-like vasculature and thus can be deleterious[83]. Also, it is unknown whether an angiogenic treatment may be sufficient to induce functional blood vessels for prolonged periods or will need to be re-administered periodically in order to maintain such vessels.

A K14-driven VEGF-C transgene induced lymphangiogenesis but no angiogenesis in mouse skin[84], and recombinant VEGF-C also stimulated lymphatic vessel hyperplasia in mature chick chorioallantoic membrane[85]. Thus, besides angiogenesis, it may also become possible to direct therapeutic lymphangiogenesis in patients, such as after evacuation of axillary lymph nodes in breast carcinoma surgery.

Despite the potential redundancy of tumor angiogenesis factors, inhibition of VEGF alone seems sufficient to achieve considerable tumor growth suppression in a wide variety of models. However, it remains to be established whether tumors are able to activate, after prolonged therapy, alternative angiogenic pathways that might confer resistance to the treatment. These issues should be addressed in the current clinical trials with various VEGF inhibitors. A challenge now in anti-VEGF (and anti-angiogenic) therapy is devising appropriate and reliable markers to monitor tumor progression. There is considerable debate whether blood vessel count in biopsy specimens[45,46] may provide a reliable indicator of response to the treatment. There are also efforts to identify surrogate endpoints, applying non-invasive approaches, such as magnetic resonance imaging[86] .

VEGF is not only a mitogen but also a potential survival factor for endothelial cells[4]. Such a 'maintenance' function seems to be developmentally regulated, as it is very dependent on the age of the animal[87]. VEGF inactivation during early postnatal life, achieved by *Cre-loxP*-mediated inducible gene targeting of by administration of a soluble VEGFR-1 chimeric protein, results in regression of the vasculature, kidney failure and lethality[87]. However, in adult animals a similar treatment has no effects on the existing vasculature. Therefore, a process of maturation occurs in endothelial cells such that VEGF eventually is not essential for survival. This switch seems to take place in the mouse around the fourth postnatal week. Absence of pericyte

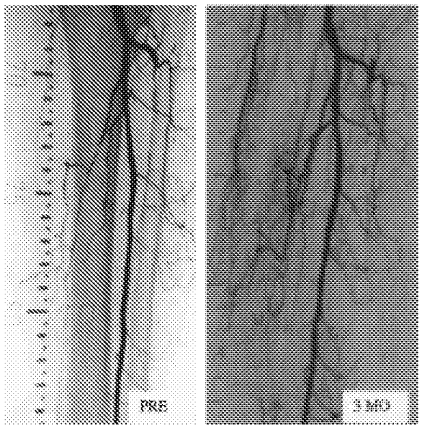


Fig. 3 Angiography of the lower extremity of a patient with limb ischemia before (PRE) and 3 months after (3 MO) the transfection of a VEGF165 plasmid/liposome expression vector, showing strongly increased vascular density after the treatment. Courtesy H. Manninen, P. Matsi, K. Mäkinen, M. Hilpeläinen, M. Laitinen, E. Alhava and S. Ylä-Herttuala, A. I. Virtanen Institute and Kuopio University Hospital (Kuopio, Finland).

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coverage in immature vessels may be a factor determining their dependence on VEGF (ref. 88). However, other evidence suggests that the molecular/intracelullar nature of this switch may be more complex and mostly still to be determined[87]. In juvenile animals, VEGF is essential for endochondral bone formation and longitudinal growth[89,55]. In the fully developed animal, VEGF may be required mainly for active angiogenic processes such as corpus luteum development or wound healing. Neverthless, VEGF may be important for endothelial homeostasis in the adult in certain circumstances; for example, during disease states. Indeed, prolonged VEGF inhibition failed to induce glomerular damage in normal primates[55] or rodents[87,90], despite the strong constitutive expression of the VEGF mRNA in podocytes and other cell types in the adult kidney[4]. However, administration of VEGF inhibitors to rats with mesangioproliferative nephritis results in impaired glomerular endothelial regeneration and increased endothelial cell death[90].

Some $CD34^+$ hematopoietic progenitor cells mobilized by GM-CSF from human peripheral blood, bone marrow, fetal liver or umbilical cord blood were shown to express VEGFR-2 on their surface[91], and VEGFR-2 is expressed on human hematopoietic stem cells[92]. Endothelial progenitor cells expand and differentiate into endothelial cells after addition of bFGF and VEGF to the cultures, and they can thus be considered to provide endothelial progenitor cells[91–93]. The endothelial progenitor cells from bone marrow may be mobilized using the stromal-derived factor 1 chemokine, the GM-CSF cytokine or tissue hypoxia[94]. As these cells may be capable of participating in active angiogenesis after entry into the circulatory system[94], they provide an interesting possibility for the delivery of cellular or gene therapy to sites of neovascularization.

Finally, the first placebo-controlled clinical study with rhVEGF may have brought a more realistic assessment of the potential of therapeutic angiogenesis and raised a number of questions. For example, how can one explain the discrepancy between the considerable efficacy observed even with very small amounts of growth factors in animal models of coronary or limb ischemia and the rather disappointing clinical results? An essential difference may lie in the fact that young and otherwise healthy animals are able to mount an effective endogenous angiogenic response that can be maximized by an additional stimulus provided by a recombinant protein or gene therapy. In contrast, patients with extensive atherosclerotic disease may have poor responses. It is possible, however, that a more persistent exposure to an individual growth factor or to a combination of growth factors may be effective. Clinical trials now ongoing should answer at least some of these questions over the next 2–3 years.

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Amelioration of Long-Term Renal Changes in Obese Type 2 Diabetic Mice by a Neutralizing Vascular Endothelial Growth Factor Antibody

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Diabetic nephropathy in type 2 diabetic patients is a frequent complication associated with increased morbidity and mortality. Various growth factors and cytokines have been implicated in the pathogenesis of diabetic kidney disease, including vascular endothelial growth factor (VEGF). To explore a role for VEGF in renal changes in type 2 diabetes, we examined the renal effects of a neutralizing murine VEGF antibody in the diabetic *db/db* mouse, a model of obese type 2 diabetes. One group of *db/db* mice was treated for 2 months with a VEGF antibody, while another *db/db* group was treated for the same period with an isotype-matched irrelevant IgG. A third group consisting of nondiabetic *db/+* mice was treated with the same isotype-matched IgG for 2 months. Placebo-treated *db/db* mice showed a pronounced increase in kidney weight, glomerular volume, basement membrane thickness (BMT), total mesangial volume, urinary albumin excretion (UAE), and creatinine clearance (CrCl) when compared with nondiabetic controls. In VEGF antibody–treated *db/db* mice, increases in kidney weight, glomerular volume, BMT, and UAE were attenuated, whereas the increase in CrCl was abolished. VEGF antibody administration tended to reduce expansion in total mesangial volume. These effects in diabetic animals were seen without impact on body weight, blood glucose, insulin levels, or food consumption. In conclusion, chronic inhibition of VEGF in *db/db* mice ameliorates the diabetic renal changes seen in type 2 diabetes. *Diabetes* 51:3090–3094, 2002

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BMT, basement membrane thickness; CrCl, creatinine clearance; LM, light microscopy; STZ, streptozotocin; TGF, transforming growth factor; UAE, urinary albumin excretion; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

The incidence of type 2 diabetes is increasing worldwide. The development of diabetic nephropathy is seen in 30–40% of type 2 diabetic individuals, with an associated increased morbidity and mortality. Accordingly, diabetic nephropathy is the most common cause of end-stage renal failure in the Western world. Mechanisms underlying the development of diabetic kidney disease in type 2 diabetes are complex. Among the many potential pathogenic mechanisms responsible for the development of diabetic kidney disease, growth factors have been suggested to be important players. Accordingly, growth hormone/IGFs and transforming growth factor (TGF)-β have been shown to have measurable effects on the development of diabetic kidney changes in animal models of type 1 diabetes (1). Recently, the vascular endothelial growth factor (VEGF) system has been proposed to play a role in the development of diabetic renal changes in animal models of type 1 diabetes (1–4); the potential role of the VEGF system in renal complications of type 2 diabetes remains unknown.

The aim of the present study was to explore the role of VEGF in the development of renal changes in type 2 diabetes. Accordingly, a specific neutralizing murine VEGF antibody was administered for 2 months in *db/db* mice, a genetic model of type 2 diabetes characterized by obesity, sustained hyperglycemia, hyperinsulinemia, lack of ketonuria, and progressive renal kidney disease (5–8).

RESEARCH DESIGN AND METHODS

Animals. Adult female *db/db* mice (C57BLKS/J-*lepr*^{db}/*lepr*^{db}) and their age-matched nondiabetic *db/+* littermates (C57BLKS/J-*lepr*^{db}/+) (M&B, Ry, Denmark) were used. Nondiabetic *db/+* mice had a body weight of 19–20 g, and the *db/db* mice had an initial weight of 39–41 g. Intervention with VEGF antibody administration was initiated at 8 weeks of age because 100% of the *db/db* mice become frankly hyperglycemic from week 7–8 (8). The *db/db* mice were included in the study 1–2 weeks after development of diabetes, at the age of 8 weeks. The mice were housed six to eight per cage in a room with a 12:12 h artificial light cycle (7:00 A.M. to 7:00 P.M.), a temperature of 21 ± 1°C, and a humidity of 55 ± 5%. The animals had free access to standard chow (Altromin no. 1324; Altromin, Lage, Germany) and tap water throughout the experiment. The study complied with Danish regulations for care and use of laboratory animals.

Study design. The *db/db* mice were randomized into two groups of 12 per group. One group of *db/db* mice was treated with intraperitoneal injections of a neutralizing VEGF antibody, and the other group was treated with an isotype-matched irrelevant IgG, as were the nondiabetic *db/+* mice (n = 6). The VEGF antibody and irrelevant IgG were administered intraperitoneally in an initial bolus dose of 300 μg, followed by doses of 100 μg three times weekly. The VEGF antibody and irrelevant IgG were dissolved in 0.154 mol/l

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TABLE 1
Mean body weight, blood glucose, and food consumption at day 0 and 60 in placebo-treated controls, placebo-treated diabetic *db/db* mice, and VEGF antibody–treated diabetic *db/db* mice

	Day 0			Day 60		
	Body weight (g)	Blood glucose (mmol/l)	Food consumption (g/24 h)	Body weight (g)	Blood glucose (mmol/l)	Food consumption (g/24 h)
Control, placebo	19.6 ± 0.4	5.4 ± 0.3	5.5 ± 0.6	21.3 ± 0.2	5.5 ± 0.3	5.9 ± 0.8
Diabetic, placebo	41.4 ± 0.7*	18.6 ± 1.0*	8.5 ± 0.7*	47.3 ± 1.0*	19.8 ± 1.4*	9.1 ± 1.1*
Diabetic, VEGF antibody	40.6 ± 0.8*	18.4 ± 1.0*	8.8 ± 0.8*	46.2 ± 0.9*	18.8 ± 2.0*	8.9 ± 0.9*

Data are means ± SE (n = 6–12 in each group). *P < 0.01 vs. nondiabetic controls.

NaCl and injected in a volume of 0.5 ml. A full characterization of the VEGF antibody used has been described elsewhere (2,4). Briefly, 8-week-old female Balb/C mice were immunized by repeated intraperitoneal and subcutaneous injections of 50 μg rhVEGF$_{165}$, which was emulsified with complete Freund's adjuvant for the primary immunization and incomplete Freund's adjuvant for the subsequent immunizations. Mice with the highest serum titer to VEGF$_{165}$ received an additional injection of 30 μg VEGF$_{165}$ in PBS, and 3 days later, spleen cells were harvested for production of hybridomas to rhVEGF$_{165}$. Two hybridoma cell lines with the highest antibody titer and neutralizing activity were cloned three to four times in microplates and injected intraperitoneally (10^7 cells). Ascites fluid was collected, and purified IgG was prepared by protein A chromatography, with a further characterization of the neutralizing activity as described previously (2).

Body weight, food consumption, and blood glucose were determined at initiation of the experiment and every 2 weeks. Blood glucose was measured in tail-vein blood as described below. After 8 weeks, mice were placed in metabolic cages to collect 24-h urine samples for urinary albumin excretion (UAE) and urinary creatinine determinations. At sacrifice, mice were anesthetized with pentobarbital (50 mg/kg i.p.) and nonfasting blood samples were drawn from the retro-orbital venous plexus using heparinized capillary tubes. Serum samples were stored at −80°C until analysis was performed. In all animals, the right and left kidneys were removed and weighed. The middle piece of the right kidney (including the papilla) was fixed in 4% paraformaldehyde for determination of glomerular volume by light microscopy (LM) (see below). The middle piece of the left kidney (including the papilla) was fixed in 0.1 mol/l cacodylate buffer with 1% glutaraldehyde and 2% paraformaldehyde for later determination of basement membrane thickness (BMT) and mesangial fraction by electron microscopy (see below). In addition, liver and heart were removed, weighed, and snap frozen in liquid nitrogen.

Determination of blood glucose and serum insulin. Blood glucose was measured at day 0 and every 2 weeks in tail-vein blood by Precision Xtra Plus (Abbott Laboratories, MediSence Products, Bedford, MA), and urine was tested for glucose and ketone bodies by Combur^5 Test D (Roche Diagnostics, Mannheim, Germany). Serum insulin was measured by an ultrasensitive rat insulin enzyme-linked immunosorbent assay (DRG Diagnostics, Marburg, Germany). Semilog linearity of mouse serum and rat insulin was found at multiple dilutions, indicating antigen similarity between mouse and rat insulin. The intra- and interassay coefficients of variation were <5% and <10% for the insulin assays.

Determination of UAE and creatinine clearance. The urinary albumin concentration was determined by radioimmunoassay as previously described (9) using rat albumin antibody and rat albumin standard. Semilog linearity of mouse urine and rat albumin (in the standard) was found at multiple dilutions, indicating antigen similarity between mouse and rat albumin. Urine samples were stored at −20° C until assay was performed. Serum and urinary creatinine concentrations were measured by an automated technique adapted from the method of Jaffé and corrected for the prevailing glucose content due to interference in the Jaffé reaction. The creatinine clearance (CrCl) was expressed in milliliters per hour. The intra- and interassay coefficients of variation were <5% and <10% for both assays.

Estimation of glomerular volume. The middle part of the right kidney (containing the papilla) was embedded in paraffin for LM examination. Two micron-thick sections were cut on a rotation microtome and stained with *p*-aminosalicylic acid and hematoxylin. The mean glomerular tuft volume (V_G) was determined from the mean glomerular cross-sectional area (A_G) at a magnification of 400×, as previously described (10–12). The areas were determined with a two-dimensional version of the nucleator (CAST; Olympus, Copenhagen, Denmark) (12) by LM as the average area of a total of 40–50 glomerular profiles (tuft omitting the proximal tubular tissue within the Bowmann capsule). V_G was calculated as $V_G = \beta/k \times (A_G)^{3/2}$, where β = 1.38, which is the shape coefficient for spheres (the idealized shape of glomeruli), and k = 1.1, which is a size distribution coefficient (10–12).

Estimation of mesangial fraction, total mesangial volume, and BMT. The middle part of the left kidney (containing the papilla) was embedded in Epon 825 for electron microscopy examination. Thin sections were cut on a Reichert Ultracut (Leica, Vienna, Austria) and stained with uranyle acetate and lead citrate. From an electron microscope (Tecnai 12; Phillips, Enthoven, Holland), images covering the whole glomerular profile were recorded with a MegaView video camera (Soft Imaging System, Münster, Germany) onto a monitor. Measurements of mesangial regions were performed at a final magnification of 3,200×. Four to six glomeruli were measured from two blocks. Mesangial fractions were determined by point counting of mesangial regions as fraction of the tuft. The total mesangial volume was calculated by multiplying the mesangial fraction by the total glomerular volume. For measurements of BMT, randomized fields were recorded at a magnification of 30,000× from the same sections described above. BMT was measured, applying the orthogonal intercept method as previously described (13). About 60 measurements were performed per glomerulus, and BMT is given as a harmonic mean.

Statistical analysis. For repeated measurements, ANCOVA was used to evaluate differences with Student's t test for unpaired comparisons. A P value <0.05 was considered statistically significant. For data not following a normal distribution, the Mann-Whitney rank-sum test was used. All data are expressed as means ± SE, with n indicating the number of mice studied. Statistical analysis was performed using SPSS for Windows.

RESULTS

Body weight, blood glucose, food consumption, and serum insulin. The *db/db* mice had a greater body weight than the nondiabetic *db/+* mice, as was also the case for food consumption (Table 1). Mean blood glucose levels were 18–19 mmol/l in *db/db* mice throughout the study, and 5–6 mmol/l in *db/+* animals (Table 1). The *db/db* mice had severe hyperinsulinemia (Table 2). VEGF antibody administration did not affect any of the above parameters in *db/db* mice throughout the study duration (Tables 1 and 2).

Kidney weight, glomerular volume, BMT, and mesangial volume. Placebo-treated *db/db* mice showed an in-

TABLE 2
Mean serum insulin, liver weight, and heart weight at day 60 in placebo-treated controls, placebo-treated diabetic *db/db* mice, and VEGF antibody–treated diabetic *db/db* mice

	Day 60		
	Serum insulin (μg/l)	Liver weight (mg)	Heart weight (mg)
Control, placebo	2.86 ± 0.29	1,136 ± 36	103 ± 4
Diabetic, placebo	18.82 ± 1.60*	2,272 ± 88*	109 ± 3
Diabetic, VEGF antibody	16.85 ± 1.67*	1,891 ± 65†	103 ± 3

Data are means ± SE (n = 6–12 in each group). *P < 0.01 vs. nondiabetic controls; †P < 0.05 vs. the two other groups.

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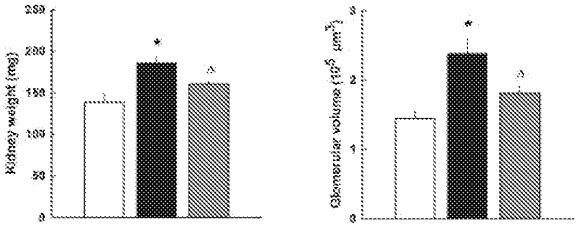


FIG. 1. Mean right kidney weight and glomerular volume on day 60 in nondiabetic controls (□), placebo-treated diabetic *db/db* mice (■), and VEGF antibody–treated diabetic *db/db* mice (▨). Values are means + SE (n = 6–12 in each group). *P < 0.01 vs. nondiabetic controls; ΔP < 0.05 vs. nondiabetic controls and placebo-treated *db/db* mice.

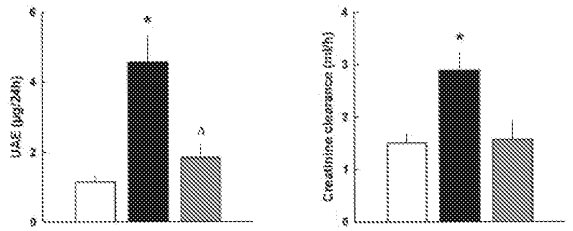


FIG. 3. Mean 24-h UAE and CrCl on day 60 in nondiabetic controls (□), placebo-treated diabetic *db/db* mice (■), and VEGF antibody–treated diabetic *db/db* mice (▨). Values are means + SE (n = 6–12 in each group). *P < 0.01 vs. nondiabetic controls; ΔP < 0.05 vs. nondiabetic controls and placebo-treated *db/db* mice.

crease in kidney weight of 34% at day 60 (187 ± 7 vs. 139 ± 11 mg, $P < 0.01$) when compared with nondiabetic *db/+* controls (Fig. 1). In VEGF antibody–treated *db/db* mice, a significantly smaller increase in kidney weight was observed versus placebo-treated *db/db* mice (161 ± 4, $P < 0.05$), although the kidney weight was higher than that seen in nondiabetic controls ($P < 0.01$). The same pattern of changes was seen in glomerular volume (Fig. 1). Total glomerular volume increased by 65% in placebo-treated *db/db* mice compared with nondiabetic controls (2.38 ± 0.22 vs. 1.44 ± 0.11 10^5 µm^3, $P < 0.01$). VEGF antibody treatment in *db/db* mice partially prevented the increase in glomerular volume versus placebo-treated *db/db* mice (1.81 ± 0.11 10^5 µm^3, $P < 0.01$). The glomerular volume was, however, still elevated above that of nondiabetic controls ($P < 0.05$). BMT increased by 18% in placebo-treated *db/db* mice when compared with nondiabetic controls (176 ± 6 vs. 149 ± 4 nm, $P < 0.05$), while an insignificant increase was seen in VEGF antibody–treated *db/db* mice (160 ± 6 nm, NS), with a value significantly lower than that of placebo-treated *db/db* mice ($P < 0.05$) (Fig. 2). Both diabetic groups had a significant increase in mesangial fraction ($P < 0.05$, data not shown), and total glomerular mesangial volume tended ($0.05 < P < 0.10$) to be lower in the VEGF antibody–treated *db/db* group (Fig. 2).

UAE and CrCl. A pronounced increase in UAE was observed in placebo-treated *db/db* mice at day 60 versus nondiabetic *db/+* controls (4.57 ± 0.75 vs. 1.15 ± 0.16 µg/24 h, $P < 0.01$), with a considerably lower level in *db/db* mice treated with the VEGF antibody (1.85 ± 0.34 µg/24 h, $P < 0.01$ vs. placebo-treated *db/db* mice) (Fig. 3). Placebo-treated *db/db* mice showed a pronounced increase in CrCl when compared with nondiabetic controls (1.51 ± 018 vs. 2.89 ± 0.35 ml/h, $P < 0.05$), with normalization in the VEGF antibody–treated *db/db* mice (1.57 ± 0.36 ml/h) (Fig. 3).

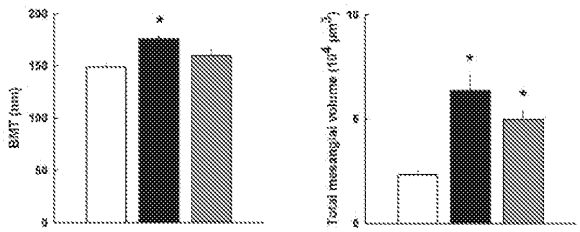


FIG. 2. BMT and total mesangial volume on day 60 in nondiabetic controls (□), placebo-treated diabetic *db/db* mice (■), and VEGF antibody–treated diabetic *db/db* mice (▨). Values are means + SE (n = 6–12 in each group). *P < 0.05 vs. nondiabetic controls and VEGF antibody–treated diabetic *db/db* mice.

Liver and heart weight. The placebo-treated *db/db* mice had greater liver weights than the *db/+* mice, whereas VEGF antibody–treated *db/db* mice had less liver weight gain (Table 2). There were no significant differences in heart weight among the three groups.

DISCUSSION

The *db/db* mouse, which expresses a leptin receptor defect in the hypothalamus, is a genetic model of type 2 diabetes characterized by obesity, sustained hyperglycemia, hyperinsulinemia, and lack of ketonuria. Previously, this model has been shown to present with robust diabetic renal changes characterized by increased renal/glomerular volume, BMT, UAE, and mesangial volume within 2 months of diabetes (5–8).

The major new finding of the present study is an amelioration of diabetes-induced renal changes in *db/db* mice by VEGF antibody administration. Accordingly, antibody administration attenuated the increase in renal/glomerular volume, BMT, and UAE and abolished the increase in CrCl. These effects were seen without affecting metabolic control, insulin levels, body weight, or food consumption, indicating that VEGF plays a causal role in the development of late renal changes in a model of type 2 diabetes.

The VEGF system consists of different isoforms of homodimeric glycoproteins (14–21). Furthermore, at least two high-affinity VEGF receptors (VEGFR-1 and -2) have been described (17). VEGF has pronounced angiogenic actions (15,18–20) and causes vasodilation and increased vascular permeability (14,18). The expression of VEGF was initially described to be markedly increased in highly vascularized rapidly growing tumors (22), and VEGF has been shown to be a potent mitogenic factor for endothelial cells (20,21). The two VEGFRs (VEGFR-1 and -2), also known as the fms-like tyrosine kinase and fetal liver kinase 1, are high-affinity transmembrane tyrosine kinase receptors (17). Both VEGF and the two VEGFRs are expressed in the kidney (3,23–27). VEGF expression and specific VEGF binding have been described in rat (23) and human kidney (24–26). VEGF has been localized to epithelial glomerular cells (i.e., podocytes) (3,26,27), distal tubules, and renal collecting ducts (3,25). Furthermore, VEGFR-2 has been localized mainly to glomerular endo-

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thelial cells and cortical interstitial fibroblasts (3). Mesangial cells, glomerular endothelial cells, vascular smooth muscle cells, and proximal and distal tubular cells are capable of producing VEGF in vitro (27–29). High glucose has been shown to stimulate VEGF expression in vascular smooth muscle cells (30). Also, in a recent study in OLETF rats (an experimental rat model of type 2 diabetes), renal VEGF mRNA and glomerular VEGF immunoreactivity were reported to be elevated over a diabetes duration of 9–68 weeks (31). In another study, changes in renal VEGF levels were described in streptozotocin (STZ)-induced diabetic rats (a rat model of type 1 diabetes) with a diabetes duration of 3 and 32 weeks (3). VEGF mRNA and protein were mainly localized to the glomerular epithelial cells and VEGFR-2 mRNA mainly to glomerular endothelial cells (3). VEGF mRNA and peptide were increased in diabetic animals at both time points examined, whereas the expression of VEGFR-2 and VEGFR binding were increased only at 3 weeks (3).

Although the area of identifying and developing specific antagonists of a pathophysiologically enhanced VEGF system in oncology and different eye diseases has attracted increasing interest (32), no studies have appeared on the effect of VEGF antagonists in diabetic kidney disease of type 2 diabetes. Direct evidence for a role of VEGF in the early renal changes observed in a model of type 1 diabetes (i.e., STZ-induced diabetic rats) has been published using the same VEGF antibody (4). Six weeks treatment with the VEGF antibody abolished the diabetes-associated hyperfiltration and partially blocked the increase in UAE (4). VEGF antibody administration in nondiabetic control rats had no impact on any renal parameters, indicating a diabetes-specific effect of VEGF antibody administration in diabetes (4). In the present study, using a mouse model of type 2 diabetes, administration of the VEGF antibody was shown to ameliorate both the classical early features of diabetic kidney disease, i.e., renal/glomerular hypertrophy and hyperfiltration (measured as CrCl), and more importantly, late renal changes (i.e., BMT), with a tendency to reduce total mesangial volume. The *db/db* mouse has previously been reported to develop decreased CrCl within 2 months after the onset of diabetes, suggesting a progressive diabetic kidney disease with loss of kidney function (8). In the present study, however, several lines of evidence indicated that placebo-treated *db/db* mice presented with renal hyperfunction, which was partially or fully normalized by VEGF antibody treatment, i.e., partial effect on kidney weight, glomerular volume, UAE, and normalization of elevated CrCl. The reason for this discrepancy is unknown, but may be explained by a variable susceptibility to diabetes in subbreedings of the *db/db* mouse strain.

The observation that VEGF antibody treatment abolished the increase in BMT and renal hyperfiltration and partially blocked the increase in UAE is interesting in view of the well-known actions of VEGF on vascular permeability (14,18) and the anatomical localization of the VEGF system in the glomerulus (i.e., podocytes and glomerular endothelial cells) (3,25–27). These results indicate that administration of a specific, neutralizing VEGF antibody in *db/db* mice fully or partly restores the abnormally increased albumin permeability in the diabetic kidney, which is believed to be caused by abnormalities in the filtration barrier due to increased membrane pore size and reduced anion charge. Although VEGF expression has been described in glomerular epithelial cells (3,26), VEGF antibody administration only tended to reduce total mesangial volume in the present study. These results suggest that the primary role of VEGF in the diabetic renal changes in type 2 diabetes is linked to the diabetes-associated permeability changes, while the role of VEGF in mesangial expansion, if any, seems to be secondary. In this context, it is interesting that administration of a neutralizing TGF-β antibody in *db/db* mice has been shown to ameliorate diabetes-associated glomerular matrix expansion without affecting either elevated UAE or renal VEGF expression (33).

Although currently unproven, several potential pathways involved in diabetes-induced vascular changes (1) may involve VEGF as a downstream cytokine. In vitro, VEGF has been shown to be stimulated by IGF-I (34), and furthermore, IGF-I receptor blockade in an ischemia-induced retinopathy model has been shown to reduce the intracellular VEGF-mediated mitogen-activated protein kinases along with ameliorating retinal neovascularization (35). Blockade of protein kinase C β activity with a specific inhibitor (LY333531) suppresses the VEGF-induced alterations in retinal leakage, retinal blood flow, and ischemia-induced retinal neovascularization (36). In addition, ACE inhibition in diabetic rats has been shown to reduce diabetes-associated retinal changes in VEGF expression and vascular permeability (37). Also, in the study described above in a rat model of type 2 diabetes (31), it was shown that long-term administration of an advanced glycation end product inhibitor (OPB-9195) abolished the enhanced renal VEGF mRNA and glomerular VEGF immunoreactivity along with renoprotection, in terms of normalization of diabetes-induced renal collagen IV accumulation and a reduction of the rise in UAE (31).

In conclusion, the present data strongly support the hypothesis that VEGF is an important pathogenetic factor in the development of long-term renal changes in type 2 diabetes. Further studies are warranted to fully elucidate the role of VEGF as a downstream mediator for some of the well-known pathways leading to diabetic renal damage.

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24. Single embryos were squished in 10 μl of 10 mM tris-HCl (pH8.0), 1 mM EDTA, and 25 mM NaCl containing Proteinase K (200 μg/ml; Boehringer Mannheim) and incubated at 37°C for 30 min, followed by 2 min at 95°C. The PCR was performed with 2.5 U of Taq polymerase and 100 ng of each primer. Two pairs of primers were designed to amplify 634 base pairs (bp) of the *crq* genomic sequence and 200 bp of the genomic region of the *doom* gene as an internal control [A. J. Harvey, A. P. Bidwaldi, L. K. Miller, *Mol. Cell. Biol.* **17**, 2835 (1997)]. *Crq*-specific primers were 5′-TGCCACCGATGCTTGCAGAT-3′ and 5′-AGCCGAATATGATTCCGTACTG-3′. *Doom*-specific primers were 5′-AGGGTAAACGGCCACAGAATGT-3′ and 5′-GATATCGTTGTAGTTGGCCCG-3′. The PCR cycles were 94°C for 1 min, 65°C for 1 min, and 72°C for 1 min for 30 cycles. In embryos from the *W88* stock, 20 of 79 were missing the *crq*-specific band.
25. CRQ immunostaining was used to genotype each embryo. Peroxidasin immunostaining detected all hemocytes [R. E. Nelson *et al.*, *EMBO J.* **13**, 3438 (1994)]. The nuclear dye 7-AAD labeled all DNA and allowed for the identification of apoptotic corpses. Unless otherwise specified, stage 11 to 16 embryos were fixed with standard procedures (*44*). Fixed devitellinized embryos were incubated in phosphate-buffered saline (PBS), 0.0125% saponin, 1% bovine serum albumin, and 4% normal goat serum (PSN) for 1 hour at room temperature and then incubated with the primary antibodies at a 1:1000 dilution in PSN overnight at 4°C. After several washes in PBS, the embryos were incubated for 1 hour at room temperature with the following secondary antibodies: fluorescein isothiocyanate–conjugated goat antibody to mouse and Cy5-conjugated goat antibody to rabbit (Jackson Immunoresearch) used at a 1:1000 dilution in PSN. Finally, embryos were washed three times in PBS for 20 min and subsequently incubated with 7-AAD (5 μg/ml) in PBS for 30 min. Embryos were quickly washed twice in PBS, mounted in Vectashield (Vector), and viewed by confocal microscopy (Leica TCS NT 4D).
26. The efficiency of engulfment was quantified by counting the number of engulfed corpses per macrophage in at least five fields of four embryos of each genotype. A P.I., that is, the mean number of engulfed corpses per macrophage, was calculated for each embryo, and the mean P.I. was derived for each genotype.
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Vessel Cooption, Regression, and Growth in Tumors Mediated by Angiopoietins and VEGF

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In contrast with the prevailing view that most tumors and metastases begin as avascular masses, evidence is presented here that a subset of tumors instead initially grows by coopting existing host vessels. This coopted host vasculature does not immediately undergo angiogenesis to support the tumor but instead regresses, leading to a secondarily avascular tumor and massive tumor cell loss. Ultimately, however, the remaining tumor is rescued by robust angiogenesis at the tumor margin. The expression patterns of the angiogenic antagonist angiopoietin-2 and of pro-angiogenic vascular endothelial growth factor (VEGF) suggest that these proteins may be critical regulators of this balance between vascular regression and growth.

It is widely accepted that most tumors and metastases originate as small avascular masses that belatedly induce the development of new blood vessels once they grow to a few millimeters in size (*1*–*3*). Initial avascular growth would be predicted for tumors that arise in epithelial structures that are separated from the underlying vasculature by a basement membrane and for experimental tumors that are implanted into avascular settings (such as the cornea pocket) or into a virtual space (such as the subcutaneum) (*2*, *3*). However, there is also evidence to suggest that tumors in more natural settings do not always originate avascularly, particularly when they arise within or metastasize to vascularized tissue (*4*). In such settings, tumor cells may coopt existing blood vessels (*4*). The interplay between this coopting of existing vessels and subsequent tumor-induced angiogenesis has not been extensively examined nor has the role of angiogenic factors in this process.

The pro-angiogenic vascular endothelial growth factors (VEGFs) and the angiopoietins are the only known growth factor families that are specific for the vascular endothelium because expression of their receptors is restricted to these cells (*5*, *6*). The angiopoietins include both receptor activators [angiopoietin-1 (Ang-1)] and receptor antagonists [angiopoietin-2

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(Ang-2)] (*7–10*). The VEGFs and the angiopoietins seem to play complementary and coordinated roles in vascular development (*9, 11*). During development, VEGF acts via the Flk1/KDR receptor to promote endothelial cell differentiation, proliferation, and primitive vessel formation (*12*). Ang-1 subsequently acts via the Tie2 receptor to remodel these primitive vessels and is then thought to help maintain and stabilize the mature vessels by promoting interactions between endothelial cells and surrounding support cells (*6–9, 11, 13, 14*). In adults, Ang-2 is expressed primarily at sites of vascular remodeling (*9, 11*), where it is thought to block the constitutive stabilizing action of Ang-1. It has been proposed that destabilization by Ang-2 in the absence of VEGF leads to frank vessel regression, whereas such destabilization in the presence of high VEGF levels facilitates the angiogenic response (*9, 11*). In tumors, hypoxia-induced VEGF (*15*) apparently recapitulates its developmental actions by contributing to the onset of tumor-associated angiogenesis, and antagonists of VEGF have been shown to inhibit the growth of many tumors (*16*).

To explore the possibility that VEGFs and angiopoietins collaborate during tumor angiogenesis, we studied early angiogenic events using the rat C6 glioma model (*17*). Remarkably, even the smallest C6 gliomas at just 1 week after implantation (<1 mm in diameter) were found to be well vascularized (Fig. 1, A and A′). As previously noted (*17*), this is attributable to the coopting of existing brain blood vessels by the implanted tumor cells. The vessels within these early tumors were similar to normal brain vessels in caliber and heterogeneity. There was no evidence of angiogenesis, as judged by the lack of vascular sprouts, noncanalized endothelial cell chains, and hyperplastic vessels. By 2 weeks after implantation, the tumors had grown to ≥2 mm in diameter but still showed no obvious angiogenic response. Rather, they exhibited a dramatic decrease in vessel density, presumably due to tumor growth in the absence of compensatory angiogenesis (Fig. 1, B and B′). The vessels within the tumors were distinctly larger and more homogeneous in caliber than the microvasculature of the normal brain. By 4 weeks after implantation, the tumors measured several millimeters in diameter and showed marked changes in comparison with tumors at earlier stages of development (Fig. 1, C and C′). Blood vessels within the core of the tumor had undergone dramatic regression, with no evidence of a local, compensatory angiogenic response. The centers of the tumors were largely bereft of vessels, leading to massive tumor cell death (Fig. 1, C and C′). The remaining cells in the tumor interior were organized in cuffs of pseudopalisading cells around the few surviving internal vessels (Fig. 1, C and C′). In contrast to the tumor interior, the tumor periphery displayed robust angiogenesis (Fig. 1, C and C′).

Regression of coopted blood vessels was a very early event that preceded tumor cell death. Apoptotic cells were predominantly found in blood vessels in early-stage tumors, whereas at later stages there was widespread apoptosis of tumor cells (Fig. 2, A through C). Staining with markers for both endothelial cells and supporting pericytes or smooth muscle cells revealed that vessel regression was associated with progressive disengagement of endothelial cells from surrounding support cells (Fig. 1, D through G).

The apparent association of tumor vessel regression, apoptosis, and disruption of endothelial cell interactions with support cells raised the possibility that blockade of the stabilizing action of Ang-1 might be contributing to tumor vessel regression. Consistent with this possibil-

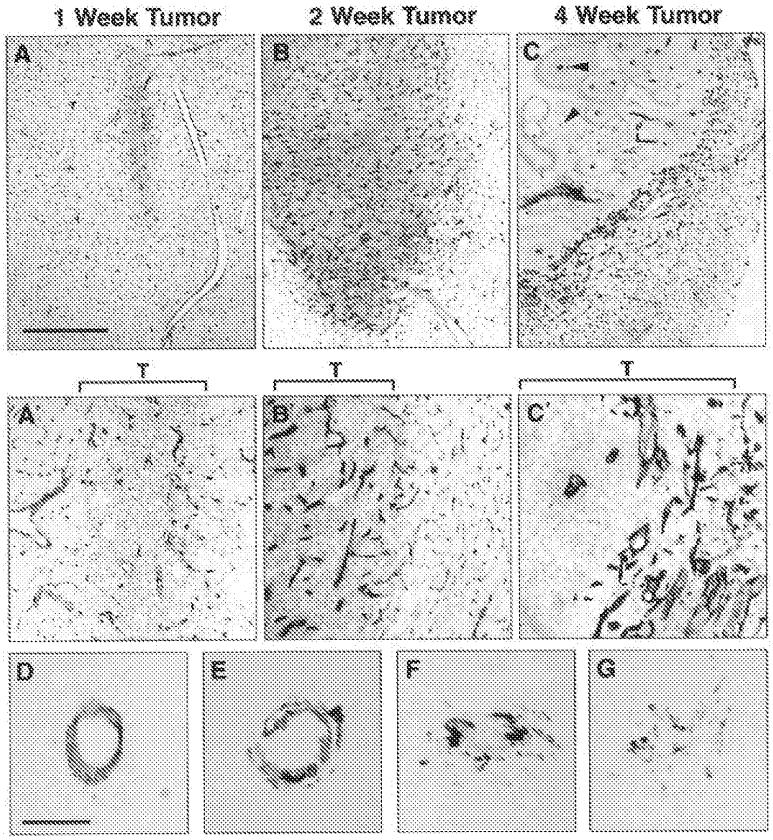


Fig. 1. Sections from rat C6 gliomas (*28*) showing progressive vessel regression, accompanied by dissociation of endothelial and smooth muscle cells. (**A** and **A′**) Small 1-week tumors that measure a fraction of a millimeter in width are well vascularized as determined by RECA immunostaining (*29*), apparently because they coopt and grow around existing vessels. [T, tumor; scale bar in (A), 1 mm for (A) through (C) and 200 μm for (A′) through (C′)] The vessels in early tumors resemble vessels in surrounding brain tissue in both density and morphology. (**B** and **B′**) Two-week tumors continue to have extensive internal vasculature, although the vessel density is less than that in surrounding brain tissue, presumably because of the growth of the tumor in the absence of compensatory angiogenesis from existing internal vessels; the caliber of the internal vessels within these tumors does become dilated and relatively uniform compared to normal brain vessels. (**C** and **C′**) Within large 4-week tumors, internal vessels regress with accompanying loss of surrounding tumor (necrotic tumor areas are unstained). Surviving internal vessels are sparse and uniform, are centrally located with respect to surrounding cuffs of well-stained viable tumor cells, and exhibit no evidence of compensatory angiogenesis; although robust angiogenesis is apparent at the margin of the tumor, where increased density of ectatic vessels is noted. Arrowheads in (C) depict a patent (top) and a regressed (bottom) vessel, each surrounded by either a surviving or regressed cuff of tumor. (**D** through **G**) Immunostaining with antibodies to SMA (black) and RECA (brown) (*29*) shows that pericytes and smooth muscle cells detach from the vessel wall in tumors. (D) shows a vessel wall in normal brain tissue in which RECA and SMA staining are essentially superimposed, whereas (E) through (G) depict vessels within tumors with progressive detachment of SMA-positive cells and vessel regression. Scale bar in (D) indicates 50 μm for (D) through (G).

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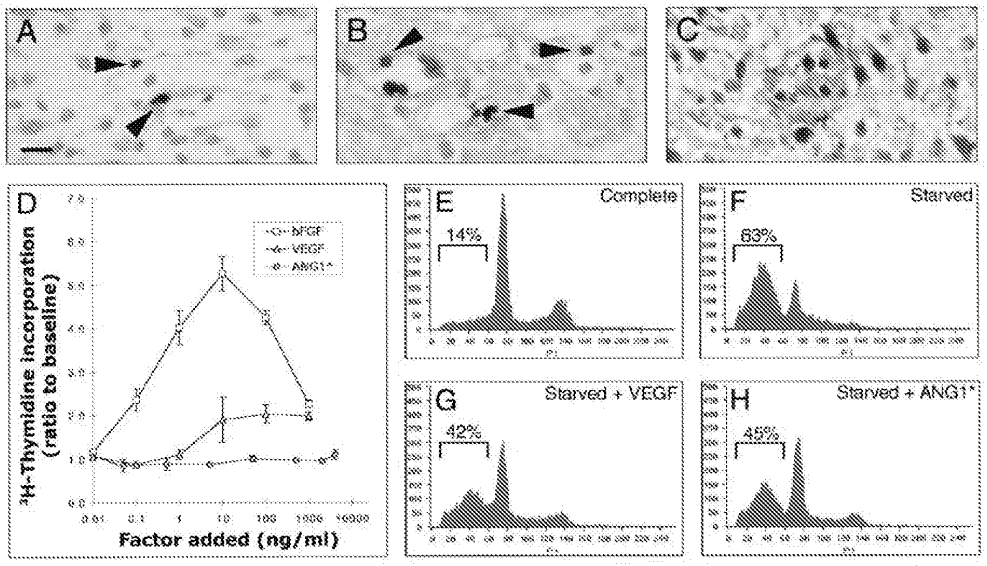


Fig. 2. Detection of apoptosis in rat C6 gliomas. Vessel-specific apoptosis (*25*, *29*) is evident in early tumors (**A** and **B**), and this is followed by widespread apoptosis of tumor cells at later stages (**C**); arrowheads denote vessel-specific apoptotic figures (stained black) in panels. Scale bar in (A), 10 μm. Flow cytometry experiments (**E** through **H**) indicate that Ang-1 can be as effective as VEGF in preventing apoptosis of serum-starved endothelial cells, as judged by a decreased percentage of endothelial cells with hypodiploid DNA content (see percentages over the sub-G_0/G_1 peak delineated by brackets). Cell number is shown on the *y* axis. PI, propidium iodide. However, in contrast to VEGF, Ang-1 cannot promote DNA synthesis in these cells (**D**) (*30*). Similar data have just been reported (*31*).

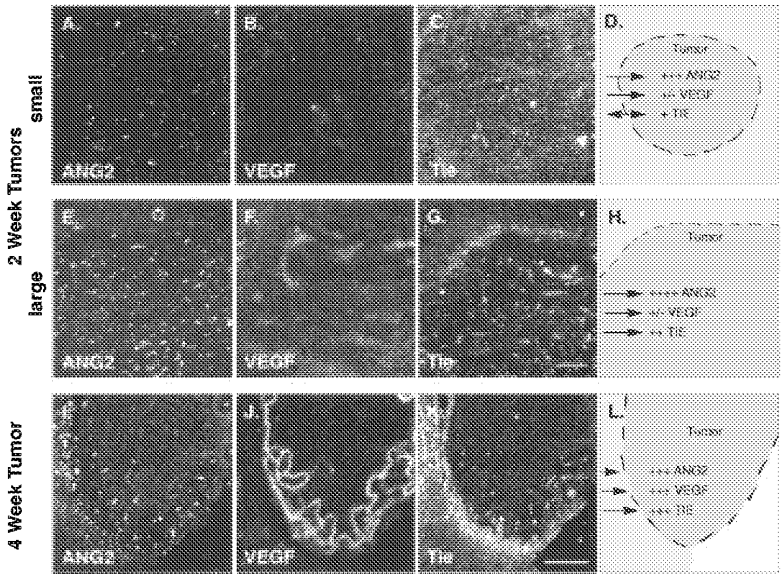


Fig. 3. In situ hybridization analysis of Ang-2, VEGF, and Tie mRNA in two different 2-week rat gliomas (small and large) and a large 4-week rat glioma (*29*, *32*). At 2 weeks, the vessels within both a small tumor (**A** through **D**) and a larger tumor (**E** through **H**) consistently express high levels of Ang-2 mRNA (A and E). In contrast, up-regulation of Tie mRNA (C and K) is restricted to the larger tumor. Induction of VEGF is minimal in small tumors (B) and is still modest and patchy in larger tumors (F). In very large 4-week tumors, the tumor is secondarily avascular because of massive vessel regression and thus has few internal vessels, but has a hypervascular plexus at the tumor border. The few internal and the many rim vessels are now marked by both Ang-2 and Tie (I and K), although expression of Ang-2 is more punctate than that of Tie. The remaining live tumor cuffs around vessels show dramatically up-regulated VEGF expression (J). This VEGF expression is highest in palisading, presumably hypoxic, tumor cells that are furthest from vessels; large areas within the tumor, between palisading cells, are necrotic. (D), (H), and (L) outline the boundaries of the tumor within the brain and indicate the relative levels of expression of Ang-2, VEGF, and Tie. Scale bar in (G) indicates 500 μm for (A) through (H); scale bar in (K) indicates 1 mm for (I) through (L).

ity, Ang-1 was found to be anti-apoptotic for cultured endothelial cells (Fig. 2, E through H), and expression of its antagonist, Ang-2, was found to be induced in the endothelium of coopted tumor vessels before their regression (Fig. 3A). In contrast, marked induction of VEGF expression occurred much later in tumor progression, in the hypoxic periphery of tumor cells surrounding the few remaining internal vessels, as well as adjacent to the robust plexus of vessels at the tumor margin (Fig. 3, B, F, and J). Expression of Ang-2 continued to mark not only the few surviving internal vessels but also the angiogenic vessels at the tumor margin (Fig. 3I), which suggests that the destabilizing action of Ang-2 facilitates the angiogenic action of VEGF at the tumor rim. Ang-1 expression did not change significantly throughout tumor development. Consistent with its expression in C6 glioma cells in culture (*18*), in relatively small tumors Ang-1 mRNA was expressed in a diffuse pattern by the tumor cells themselves (*19*) at levels just above that in the normal brain. Unlike VEGF, Ang-1 was not expressed at elevated levels in hypoxic regions of large tumors (*20*–*22*).

We also examined human glioblastomas (*20*, *21*). Ang-2 was not detectable in the normal human brain, but its expression was dramatically induced in coopted tumor vessels, preceding vessel regression. As in the rat C6 model, this occurred in association with a disruption of interactions between endothelial and smooth muscle cells and with endothelial cell apoptosis. Diffuse Ang-1 expression in the human tumors also resembled that seen in the rat model (*20*–*22*).

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To examine whether these findings are generalizable to other tumor types, we implanted rat RBA mammary adenocarcinoma cells into rat brains. Rather than growing avascularly, the implanted RBA cells rapidly associated with and migrated along cerebral blood vessels in a manner even more striking than that observed with the glioma cells (Fig. 4, A and D). Consistent with the well-vascularized state of these early tumors, there was minimal up-regulation of VEGF (*22*). However, the coopted vessels displayed striking and specific up-regulation of Ang-2, which was not detectable in the vessels of adjacent brain tissue (Fig. 4B). Preliminary analysis of RBA tumors at a later stage indicated that Ang-2 expression was associated with a pattern of vascular regression (in the absence of VEGF) and angiogenesis (in the presence of VEGF), as was the case with gliomas (*22*). Ang-1 was not expressed in cultured RBA cells or the tumors themselves (*22*).

Examination of a model of tumor metastasis, in which the mouse lung is colonized by intravenously injected Lewis lung carcinoma cells, yielded similar results. Tiny tumor metastases (arrowheads, Fig. 4, E and F) as well as moderately sized tumor nodules (arrows, Fig. 4, E and F) were closely associated with pulmonary vessels, and these vessels showed dramatic induction of Ang-2 expression (Fig. 4F). Progressively larger tumor nodules appeared to be characterized by vessel regression as well as neo-angiogenesis, again correlating with Ang-2 and VEGF expression (*22*).

In summary, our analyses of several different tumor models suggest a modification of the prevailing view that most malignancies and metastases originate as avascular masses that only belatedly induce angiogenic support. Our findings indicate that a subset of tumors rapidly coopts existing host vessels to form an initially well-vascularized tumor mass. Perhaps as part of a host defense mechanism, there is widespread regression of these initially coopted vessels, leading to a secondarily avascular tumor and massive tumor cell loss; however, the remaining tumor is ultimately rescued by robust angiogenesis at the tumor margin.

The expression patterns of VEGF and the natural Tie2 receptor antagonist Ang-2 strongly implicate them in these processes. There is a striking induction of Ang-2 expression in coopted vessels before induction of VEGF expression in the adjacent tumor cells, providing perhaps the earliest marker of tumor vasculature. The intense autocrine expression of Ang-2 by endothelial cells in tumor-associated vessels may counter a paracrine stabilization or survival signal provided by low-level constitutive expression of Ang-1 in normal tissues. We hypothesize that Ang-2 "marks" the coopted vessels for regression by an apoptotic mechanism that may involve disrupted interactions between endothelial cells and the surrounding extracellular matrix and supporting cells. Subsequently, VEGF up-regulation coincident with Ang-2 expression at the tumor periphery is associated with robust angiogenesis. This late expression of tumor-derived VEGF may nullify the regression signal provided by Ang-2, which is consistent with the observation that VEGF is required for tumor vessel survival (*23*).

The angiogenic properties of tumor-derived VEGF may actually be facilitated when vessels are destabilized by Ang-2. Newly formed tumor vessels are often tenuous, poorly differentiated, and undergo regressive changes even as blood vessel proliferation continues. The failure of many solid tumors to form a well-differentiated and stable vasculature may be attributable to the fact that newly formed tumor vessels continue to overexpress Ang-2. In fact, hypervascular hepatomas with aberrant vasculatures show high levels of Ang-2 expression in their endothelium (*24*). Thus, a persistent blockade of Tie2 signaling, which is otherwise constitutively activated in many normal adult tissues (*14*), may prevent tumor vessel differentiation and maturation and contribute to their generally tenuous and leaky nature.

In tumors, Ang-2 and VEGF apparently reprise the roles they play during vascular remodeling in normal tissues, acting to regulate the previously underappreciated balance between vascular regression and growth. Our findings bolster the case for anti-VEGF therapies in cancer, not only to prevent further angiogenesis but also perhaps to promote the regression of fragile new tumor vessels. Ang-2 appears to be the earliest marker of blood vessels that have been perturbed by invading tumor cells. As such, Ang-2 may prove to be useful in the imaging of very

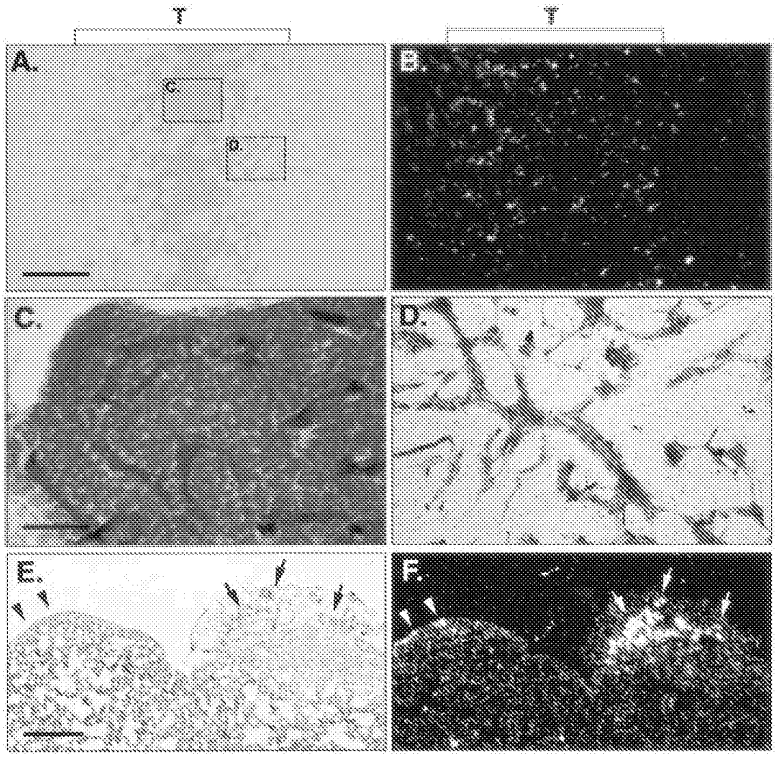


Fig. 4. In situ hybridization analysis (*32*) of rat RBA mammary carcinomas (*28*) and mouse Lewis lung carcinomas (*28*), showing up-regulation of Ang-2 mRNA in coopted tumor vessels. (**A**) A section through a mammary carcinoma stained with cresyl violet demonstrates the invasiveness of the tumor cells in the brain. The boxes within (A) delineate regions of the tumor core and periphery. Similar regions in specimens stained with an antibody to RECA (*29*) are shown in (**C**) and (**D**). The dramatic homing of tumor cells to blood vessels is especially apparent in (D). (**B**) Whereas VEGF is typically weak or undetectable at this tumor stage (*22*), Ang-2 is highly expressed in a punctate manner by blood vessels. (**E**) A section through a Lewis lung carcinoma stained with Pyronin Y demonstrates a metastasis only a few cells thick (left, arrowheads) and a slightly larger metastasis (right, arrows). Vessels, stained black with antibodies to PECAM (*29*), lie within these small tumors. (**F**) The vessels coopted by the small Lewis lung metastases exhibit dramatic induction of Ang-2. Scale bar in (A) indicates 500 μm for (A) and (B); scale bar in (C) indicates 25 μm for (C) and 50 μm for (D); scale bar in (E) indicates 500 μm for (E) and (F).

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small tumors and metastases and possibly in schemes designed to specifically target chemotoxic therapy to tumor vasculature.

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28. Tumor cells were obtained from the American Type Culture Collection and grown in culture. About 1.0×10^5 C6 or RBA cells were suspended in ~2 μl of phosphate-buffered saline (PBS) and injected stereotaxically over a period of 5 to 10 min into the right striatum (AP + 0.5; ML −3.0; DV −6.0 relative to Bregma) of adult male Sprague-Dawley rats. About 5.0×10^5 Lewis lung carcinoma cells were suspended in 50 μl of serum-free media and injected into the jugular vein of adult male C57Bl mice.
29. Animals were anesthetized and either decapitated or perfused with 4% paraformaldehyde. Brains for thick sliding microtome sections (40 μm) were post-fixed in 4% paraformaldehyde overnight and then equilibrated in 35% sucrose. Fixed brains for thin sections (10 μm) were equilibrated in 17% sucrose. Fixed and fresh brains were frozen in methylbutane, chilled in dry ice, and sectioned on a cryostat. For TUNEL labeling (*25*), brains were immersion-fixed in 10% neutral buffered formalin and embedded in paraffin. Fixed sections were immunostained with a monoclonal antibody to rat endothelial cell antigen (RECA1; 1:250; Serotec) and a biotinylated horse anti-mouse secondary antibody (1:1500; Vector) or with a monoclonal antibody to PECAM (CD31; 1:100; Pharmingen) and a biotinylated rabbit anti-rat secondary antibody (1:150; Vector) as previously described (*26*). A similar protocol was used for double labeling. Sections were initially labeled with a monoclonal antibody to alpha smooth muscle actin (SMA; 1:500; DAKO) and a biotinylated goat anti-mouse immunoglobulin G IIa secondary antibody (1:1250; Amersham). SMA staining was visualized with a Vectastain Elite kit (Vector), and a black reaction product was generated by nickel sulfate enhancement. After SMA labeling, sections were then reblocked and labeled with antibody to RECA (1:100). A brown reaction product was used.
30. Human umbilical vein endothelial cells (HUVECs) (Clonetics, San Diego, CA) were maintained in recommended medium on gelatin-coated plastic. For DNA synthesis assays, 1×10^4 cells were plated in 96-well microwells and grown for 24 hours in basal medium plus 0.5% fetal bovine serum. Cells were re-fed with the same medium plus purified factors and grown for 20 hours, with 1 mCi tritiated thymidine (80 Ci/mmol; Amersham) being present for the last 3 hours of incubation. Cells were rinsed and fixed with trichloroacetic acid, and thymidine incorporation was measured by standard liquid scintillation techniques. Ang-1* (ANG1*) was a modified form of human Ang-1, described previously (*9*); VEGF was murine VEGF-164, produced and purified from baculovirus-infected insect cells; bFGF was human basic fibroblast growth factor (R&D Systems). For assessing resistance to apoptosis, plates of ~80% confluent HUVECs were rinsed twice with basal medium and grown for 18 to 20 hours in basal medium and bovine serum albumin (0.5 mg/ml), plus or minus purified factors. Both adherent and nonadherent cells were harvested, pooled, and fixed in 70% ethanol at −20°C overnight. Cells were washed in PBS, incubated for 30 min with ribonuclease A (5 kunitz units/ml; Sigma) and propidium iodide (50 μg/ml; Sigma). Cellular DNA content, as judged by propidium iodide fluorescence, was measured by flow cytometry (MoFlo, Cytomation, Fort Collins, CO).
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32. Fresh frozen or fixed sections were probed with ^{35}S-labeled cRNAs (*27*). Probes for VEGF and Ang-1 and Ang-2 have been described (*9*). For Tie1, a 1.3-kb fragment of rat Tie1 spanning the last 309 codons and 375 base pairs of the 3′ untranslated sequence was used, and for Tie2 a 460–base pair fragment spanning codons 771 through 924 within the kinase domain was used. This probe does not cross-hybridize to Tie1 mRNA in Northern blots.

33. We thank B. Luan, J. Zheng, P. Burfeind, S. Zabski, and F. Martin for excellent technical assistance; E. Burrows and C. Murphy for graphics work; A. Hooper and D. Friedlander for data on human gliomas; and M. Grumet for intellectual discussions. Supported in part by a grant from the Children's Brain Tumor Foundation to D.Z. and by Procter & Gamble Pharmaceuticals, Inc. All animal studies were done in accordance with institutional guidelines.

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Initiation of Mammalian Liver Development from Endoderm by Fibroblast Growth Factors

Joonil Jung,[1] Minghua Zheng,[1] Mitchell Goldfarb,[2] Kenneth S. Zaret[1]*

The signaling molecules that elicit embryonic induction of the liver from the mammalian gut endoderm or induction of other gut-derived organs are unknown. Close proximity of cardiac mesoderm, which expresses fibroblast growth factors (FGFs) 1, 2, and 8, causes the foregut endoderm to develop into the liver. Treatment of isolated foregut endoderm from mouse embryos with FGF1 or FGF2, but not FGF8, was sufficient to replace cardiac mesoderm as an inducer of the liver gene expression program, the latter being the first step of hepatogenesis. The hepatogenic response was restricted to endoderm tissue, which selectively coexpresses FGF receptors 1 and 4. Further studies with FGFs and their specific inhibitors showed that FGF8 contributes to the morphogenetic outgrowth of the hepatic endoderm. Thus, different FGF signals appear to initiate distinct phases of liver development during mammalian organogenesis.

Identifying the molecular signals that initiate organogenesis from the gut is important for understanding the fundamental mechanisms of developmental regulation, hereditary digestive disorders, and tissue regeneration. Different segments of the mammalian gut endoderm give rise to the liver, lung, pancreas, thyroid, and gastrointestinal tract. Typically, a portion of the endoderm will begin to express genes specific to one of these tissues, and then the newly specified cells will proliferate out of the endoderm layer to form a tissue bud, initiating morphogenesis (*1, 2*). In *Drosophila*, the initial specification of tissues

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Vessel Cooption, Regression, and Growth in Tumors Mediated by Angiopoietins and VEGF

J. Holash, P. C. Maisonpierre, D. Compton, P. Boland, C. R. Alexander, D. Zagzag, G. D. Yancopoulos and S. J. Wiegand

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Intravitreal Aflibercept Injection for Macular Edema Resulting from Central Retinal Vein Occlusion

One-Year Results of the Phase 3 GALILEO Study

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Purpose: To evaluate the efficacy and safety of intravitreal aflibercept injections for treatment of macular edema secondary to central retinal vein occlusion (CRVO).

Design: A randomized, multicenter, double-masked phase 3 study.

Participants: A total of 177 treatment-naive patients with macular edema secondary to CRVO were randomized in a 3:2 ratio.

Methods: Patients received either 2-mg intravitreal aflibercept or sham injections every 4 weeks for 20 weeks. From week 24 to 48, the aflibercept group received aflibercept as needed (pro re nata [PRN]), and the sham group continued receiving sham injections.

Main Outcome Measures: The primary efficacy end point was the proportion of patients who gained 15 letters or more in best-corrected visual acuity (BCVA) at week 24. This study reports week 52 results including the proportion of patients who gained 15 letters or more in BCVA and the mean change from baseline BCVA and central retinal thickness. Efficacy end points at week 52 were all exploratory.

Results: At week 52, the mean percentage of patients gaining 15 letters or more was 60.2% in the aflibercept group and 32.4% in the sham group ($P = 0.0004$). Aflibercept patients, compared with sham patients, had a significantly higher mean improvement in BCVA (+16.9 letters vs. +3.8 letters, respectively) and reduction in central retinal thickness (−423.5 μm vs. −219.3 μm, respectively) at week 52 ($P < 0.0001$ for both). Aflibercept patients received a mean of 2.5 injections (standard deviation, 1.7 injections) during PRN dosing. The most common ocular adverse events in the aflibercept group were related to the injection procedure or the underlying disease, and included macular edema (33.7%), increased intraocular pressure (17.3%), and eye pain (14.4%).

Conclusions: Treatment with intravitreal aflibercept provided significant functional and anatomic benefits after 52 weeks as compared with sham. The improvements achieved after 6 monthly doses at week 24 largely were maintained until week 52 with as-needed dosing. Intravitreal aflibercept generally was well tolerated. *Ophthalmology 2014;121:202-208* © *2014 by the American Academy of Ophthalmology.*



**Group members listed online in Appendix 1 (http://aaojournal.org).*

The most common cause of vision loss in patients with central retinal vein occlusion (CRVO) is macular edema, which resolves spontaneously in only 30% of nonischemic cases and may not resolve in ischemic cases.[1,2] Several lines of evidence indicate that vascular endothelial growth factor (VEGF) may play a key role in the pathophysiology of macular edema secondary to CRVO. Vascular endothelial growth factor is released in response to retinal hypoxia, which occurs in CRVO as a result of impaired capillary blood flow.[3] Vascular endothelial growth factor stimulates angiogenesis and may result in neovascularization of the retina, the anterior segment, or both, as well as vascular leakage resulting in macular edema.[3] In CRVO patients, the vitreous level of VEGF correlates with the severity of macular edema.[4] Furthermore, intravitreal injections of the anti-VEGF agents ranibizumab or aflibercept significantly improve visual and anatomic outcomes in patients with macular edema secondary to CRVO.[5–9]

Intravitreal aflibercept (historically known in the scientific literature as VEGF Trap-Eye; Regeneron Pharmaceuticals, Inc, Tarrytown, NY, and Bayer Healthcare Pharmaceuticals, Berlin, Germany) is a fusion protein of key domains from human VEGF receptors 1 and 2 with the

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constant region (Fc) of human immunoglobulin G that binds to multiple VEGF-A isoforms with a higher affinity than ranibizumab and bevacizumab.[10] Studies of intravitreal aflibercept injections in patients with neovascular age-related macular degeneration (AMD) demonstrate that aflibercept given monthly for 3 initial administrations and then once every 2 months improves visual and anatomic outcomes as effectively and safely as monthly ranibizumab over a 1-year period.[11] The efficacy and safety of intravitreal aflibercept for the treatment of macular edema secondary to CRVO was investigated in 2 parallel trials performed in Europe and in the Asia Pacific region (GALILEO) and in the United States (COPERNICUS).[5,7,9] The primary efficacy end point of the GALILEO study was at week 24 and was published previously.[9] Herein, we report the 52-week results of the GALILEO study.

Methods

Study Design

The GALILEO study is an 18-month, randomized, double-masked, phase 3 study comparing the efficacy and safety of intravitreal aflibercept with sham for the treatment of macular edema secondary to CRVO. The study protocol was approved by the institutional review board or ethics committee at each site. All patients signed a written consent form before initiation of the study-specific procedures. The study was registered with ClinicalTrials.gov (identifier no. NCT01012973) and was conducted across 63 sites in Europe and the Asia Pacific region in compliance with ethical guidelines from the Declaration of Helsinki and International Conference on Harmonization. Data for this 52-week report were collected between October 2009 and July 2011.

The design and eligibility criteria for the GALILEO study have been described previously.[9] Only 1 eye from each patient was included in the study. Patients were randomized in a 3:2 ratio to receive 2 mg intravitreal aflibercept (IVT-AFL 2Q4) or sham injections in the study eye once every 4 weeks for 20 weeks, for a total of 6 doses (Fig 1). From weeks 24 to 52, patients in the aflibercept group were evaluated monthly and received aflibercept as needed (pro re nata [PRN]; IVT-AFL 2Q4 + PRN) if they had more than a 50-μm increase in central retinal thickness (CRT) compared with the lowest previous measurement, new or persistent cystic changes within the neurosensory retina or subretinal fluid, persistent diffuse edema of 250 μm or more in the central subfield, loss of 5 letters or more from the best prior measurement in conjunction with any increase in CRT, or an increase of 5 letters or more in best-corrected visual acuity (BCVA) from the most recent visit, potentially suggesting further improvements on a subsequent injection. If none of the retreatment criteria were met, patients received a sham injection to maintain masking. Patients in the sham group continued to receive sham injections at all visits through week 52. All patients were eligible to receive panretinal laser photocoagulation at any time during the study if they progressed to neovascularization of the anterior segment, optic disc, or elsewhere in fundus. Given that there was no approved treatment for CRVO when the GALILEO study was designed, no other rescue treatment was prespecified. The GALILEO study design included a full year of treatment with sham based on the request from health authorities. However, considering this long duration of sham treatment, the visual acuity and other ocular findings were monitored carefully by a team of masked medical reviewers. If, at any time, this review team had the impression that a patient may not benefit from further study participation or would be treated more adequately outside the study, the investigator was queried and asked to provide a reassessment of the patient. Investigators then used their medical judgment ultimately to determine whether it would still be beneficial for the patient to continue the study.

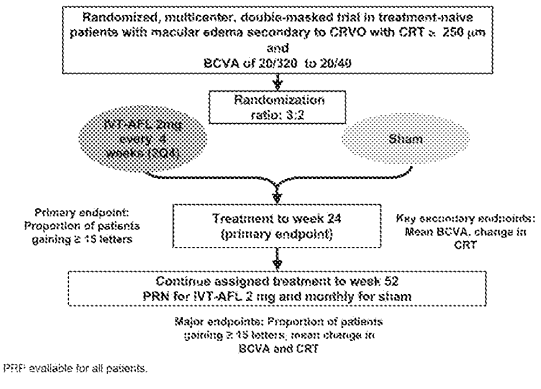


Figure 1. Diagram showing the GALILEO study design. BCVA = best-corrected visual acuity; CRT = central retinal thickness; CRVO = central retinal vein occlusion; IVT-AFL = intravitreal aflibercept; PRN = pro re nata (as needed); PRP = panretinal photocoagulation; 2Q4 = every 4 weeks.

Outcome Measures

The primary efficacy end point of the GALILEO study was the proportion of patients achieving a gain of 15 letters or more in BCVA from baseline to week 24, which was published previously.[9] Herein, we report the 52-week results of the GALILEO study. Efficacy end points at week 52 all were exploratory and included the proportion of patients who gained 15 letters or more in BCVA; mean change from baseline BCVA and CRT; proportion of patients progressing to neovascularization of the anterior segment, optic disc, or elsewhere in the fundus; and change from baseline in the mean 25-item National Eye Institute Visual Function Questionnaire total and subscale (distance activities, near activities, and vision dependency) scores.

The efficacy and safety end points were assessed as described previously.[9] The BCVA and CRT were assessed at baseline and every 4 weeks afterward to week 52. Fundus photography and fluorescein angiography were performed at screening (days −21 to −1) and weeks 12, 24, 36, and 52. Retinal perfusion status was determined by fluorescein angiography. Perfused and nonperfused retinas were defined as those with less than 10 disc areas and 10 disc areas or more, respectively, of capillary nonperfusion on fluorescein angiography. Vision-related quality of life was assessed at baseline and weeks 24 and 52 using the 25-item National Eye Institute Visual Function Questionnaire, which was administered by masked site personnel before intravitreal injections.

Statistical Analyses

The efficacy end points were analyzed in the full analysis set (FAS), which included all randomized patients who received any study treatment and had a baseline and at least 1 postbaseline BCVA assessment. In a prespecified analysis of proportions of patients who gained 15 letters or more at week 24 (the primary efficacy end point), patients who discontinued before week 24 were

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considered to be nonresponders. In a prespecified analysis of proportions of patients who gained 15 letters or more at week 52, the missing values were imputed by the last-observation-carried-forward method. Between-group differences in the proportion of patients who gained 15 letters or more were evaluated with a 2-sided Cochran-Mantel-Haenszel test.

Continuous variables were analyzed with an analysis of covariance, except for BCVA, which was assessed using an analysis of variance. The last-observation-carried-forward approach was used to impute missing values. For sensitivity, additional analyses were performed using observed values at week 52. The proportion of patients with neovascularization by week 52 was analyzed using a Cochran-Mantel-Haenszel test. Safety from baseline to week 24 was analyzed in the safety analysis set, which included all randomized patients who received any study treatment. Safety from weeks 24 to 52 was analyzed in week 24 completers within the safety analysis set.

Results

Of 240 patients screened, 106 patients were randomized to the IVT-AFL 2Q4 + PRN group, and 71 patients were randomized to the sham group. A total of 104 (98.1%) patients in the IVT-AFL 2Q4 + PRN group and 68 (95.8%) patients in the sham group were treated in the study and were included in the safety analysis set. One patient did not have any postbaseline BCVA value, and therefore was excluded from the FAS. Thus, the FAS included 103 patients in the IVT-AFL 2Q4 + PRN group and 68 patients in the sham group. Overall, 15 (14.2%) patients in the IVT-AFL 2Q4 + PRN group and 19 (26.8%) patients in the sham group discontinued the study before week 52. Major reasons for discontinuation in the IVT-AFL 2Q4 + PRN group were protocol violation (5 patients [4.7%]), withdrawal of consent (4 patients [3.8%]), and adverse events (4 patients [3.8%]). Major reasons for discontinuation in the sham group were lack of efficacy (6 patients [8.5%]), withdrawal of consent (6 patients [8.5%]), and adverse events (4 patients [5.6%]). No patient in the IVT-AFL 2Q4 + PRN group discontinued the study treatment because of a lack of efficacy.

Demographics and baseline disease characteristics of patients were similar in both treatment groups.[9] Approximately half of patients had CRVO for less than 2 months (53.4% in the IVT-AFL 2Q4 + PRN group and 51.5% in the sham group, FAS). Most patients had a perfused retina (86.4% in the IVT-AFL 2Q4 + PRN group and 79.4% in the sham group) and a baseline BCVA of 35 letters or better (>20/200; 83.5% in the IVT-AFL 2Q4 + PRN group and 82.4% in the sham group).[9]

Visual Outcomes

At week 24, the proportion of patients who gained 15 letters or more in BCVA was 60.2% and 22.1% in the IVT-AFL 2Q4 and sham groups, respectively (patients who discontinued before week 24 were considered to be nonresponders; $P < 0.0001$).[9] At week 52, the proportion of patients who gained 15 letters or more in BCVA was 60.2% in the IVT-AFL 2Q4 + PRN group versus 32.4% in the sham group (last observation carried forward; Fig 2A). More patients in the sham group had 15 letters or more of improvement in BCVA at week 52 compared with week 24 (32.4% vs. 22.1%, respectively). At week 52, patients treated with IVT-AFL 2Q4 + PRN maintained the improvements in BCVA achieved at week 24.

The proportion of patients who gained 10 or more letters and 30 or more letters or those who lost more than 0, more than 10, and more than 15 letters at week 52 are shown in Table 1. Overall, higher proportions of sham patients lost more than 0, more than 10, and more than 15 letters compared with patients treated with IVT-AFL 2Q4 + PRN at week 52 (Table 1).

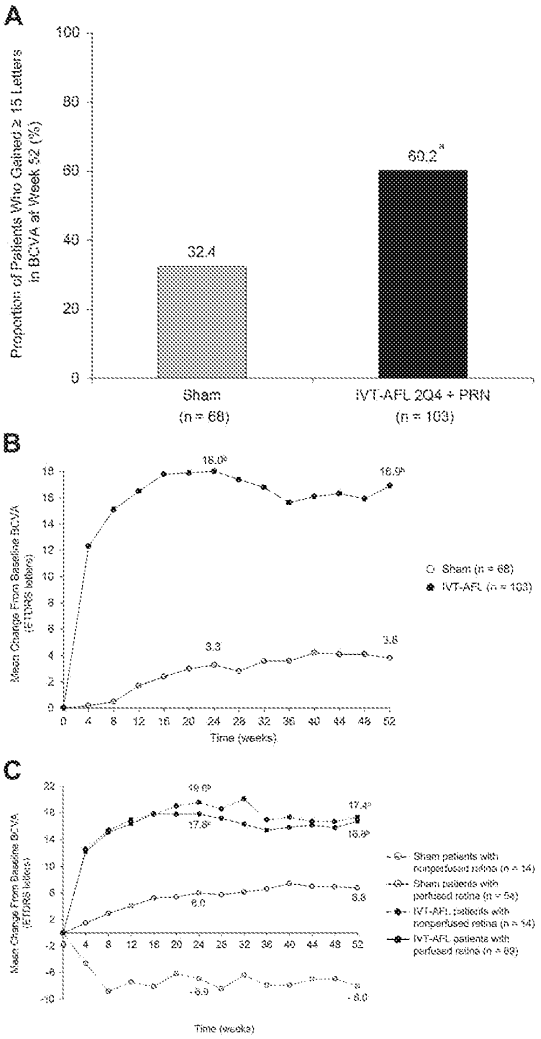


Figure 2. Graphs showing visual outcomes during the 52 weeks of the study: (**A**) percentage of patients who gained 15 letters or more at week 52, (**B**) mean change from baseline best-corrected visual acuity (BCVA), and (**C**) mean change from baseline BCVA by the status of retinal perfusion at baseline. Treatment frequency with intravitreal aflibercept (IVT-AFL) was every 4 weeks (2Q4) and pro re nata (PRN; as needed), respectively, before and after week 24. [a]$P = 0.0004$ vs. sham; [b]$P < 0.0001$ vs. sham; [c]$P < 0.001$ vs. sham. ETDRS = Early Treatment Diabetic Retinopathy Study.

The mean change from baseline BCVA in the IVT-AFL 2Q4 + PRN and sham groups was 18.0 versus 3.3 letters at week 24 and 16.9 versus 3.8 letters at week 52 ($P < 0.0001$ for both; Fig 2B). When stratified by the baseline retinal perfusion status, patients treated with IVT-AFL 2Q4 + PRN had a similar mean ± standard deviation (SD) change from baseline BCVA in the perfused and nonperfused subgroups (+16.8±14.7 letters vs. +17.4±16.1

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Table 1. Patients with Vision Gain and Loss at Week 52

	Week 52	
	Sham (n = 68)	*Intravitreal Aflibercept Injection Monthly from Baseline to Week 24 plus Pro Re Nata Treatment from Weeks 24 to 52 (n = 103)*
Vision gain, n (%)		
≥30 letters	5 (7.4)	15 (14.6)
≥15 letters	22 (32.4)	62 (60.2)
≥10 letters	26 (38.2)	74 (71.8)
Vision loss, n (%)		
>0 letters	30 (44.1)	11 (10.7)
>10 letters	16 (23.5)	1 (1.0)
>15 letters	10 (14.7)	1 (1.0)

letters, respectively; Fig 2C). In contrast, eyes with a perfused retina in the sham group gained a mean ± SD of 6.8±17.5 letters, whereas those with a nonperfused retina lost a mean of 8.0±15.8 letters at 52 weeks (Fig 2C). Regardless of the treatment group, patients with a baseline BCVA of 20/200 or worse had a greater BCVA gain than those with a baseline BCVA of better than 20/200 (9.4 vs. 2.5 letters for sham and 21.1 vs. 16.0 letters for IVT-AFL 2Q4 + PRN, respectively). Patients who had the disease for less than 2 months in the sham and IVT-AFL 2Q4 + PRN groups gained a mean of 2.1 letters and 19.5 letters from baseline, respectively, whereas those having the disease for 2 months or more gained a mean of 5.5 letters and 13.7 letters from baseline, respectively.

Anatomic Outcomes

At week 24, the mean CRT reduction from baseline was 448.6 μm and 169.3 μm in the IVT-AFL 2Q4 and sham groups, respectively ($P < 0.0001$). With the start of PRN dosing at week 24, CRT slightly increased in the IVT-AFL 2Q4 + PRN group, but then remained stable through week 52 (Fig 3). At week 52, the mean CRT reduction from baseline was significantly greater in the IVT-AFL 2Q4 + PRN group than in the sham group (423.5 μm vs. 219.3 μm, respectively; $P < 0.0001$). Regardless of the retinal perfusion status, patients treated with IVT-AFL 2Q4 + PRN had a greater CRT reduction (±SD) than those treated with sham (412.4±238.1 μm vs. 201.2±226.4 μm for the perfused subgroup and 494.6±318.4 μm vs. 294.3±258.6 μm for the nonperfused subgroup, respectively). During the 52-week study, 6 (5.8%) patients in the IVT-AFL 2Q4 + PRN group and 6 (8.8%) patients in the sham group developed neovascularization. In each group, 3 patients had a nonperfused retina at baseline, and 5 had disease duration of less than 2 months at baseline. In the IVT-AFL 2Q4 + PRN group, 4 patients demonstrated anterior segment neovascularization, 1 patient demonstrated neovascularization elsewhere in the fundus, and 1 patient demonstrated neovascularization both in anterior segment and elsewhere in the fundus. In the sham group, 4 patients demonstrated neovascularization of elsewhere in the fundus, 1 patient demonstrated anterior segment neovascularization, and 1 patient demonstrated neovascularization of optic disc. Panretinal photocoagulation was performed for 3 (4.4%) of the sham patients and 2 (1.9%) of the IVT-AFL 2Q4 + PRN patients.

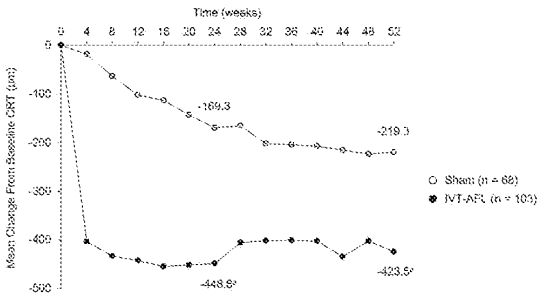


Figure 3. Graph showing the mean change from baseline central retinal thickness (CRT) during the 52 weeks of the study. Treatment frequency with intravitreal aflibercept (IVT-AFL) was every 4 weeks and pro re nata (as needed), respectively, before and after week 24. [a]$P<0.0001$ vs. sham.

Patient-Reported Outcomes

A clinically relevant improvement in the mean 25-item National Eye Institute Visual Function Questionnaire total score (≥4-point increase) was observed in both IVT-AFL 2Q4 + PRN group (7.8 points) and sham group (4.5 points) at week 52 (Table 2). The mean change from baseline to week 52 in near activities subscore was the highest among subscales, with IVT-AFL 2Q4 + PRN patients reporting a mean change of 12.2 points versus sham patients reporting a mean change of 5.0 points. No difference was noted between the 2 groups in the dependency subscale.

Study Drug Injections

During the 52 weeks of treatment, the mean (±SD) number of injections was 11.8±2.8 in the IVT-AFL 2Q4 + PRN group and 10.5±4.2 in the sham group. Most IVT-AFL 2Q4 + PRN patients (64 of 91 patients completing week 52 [70.3%]) received 3 or fewer IVT-AFL injections during weeks 24 to 52, with a mean ± SD of 2.5±1.7 injections during the PRN phase of study (Table 3). Patients who received 3 PRN injections or fewer had relatively higher BCVA gains than those who received 4 to 6 injections (Table 3). The median time to the first PRN intravitreal aflibercept injection was 83 days (95% confidence interval, 62–88 days).

Safety

The percentage of patients experiencing at least 1 ocular treatment-emergent adverse event (TEAE) in the sham and intravitreal aflibercept groups was 64.7% and 54.8% from baseline to week 24 and 50.9% and 69.1% from week 24 to week 52, respectively. The most common ocular TEAEs reported for the study eye in the intravitreal aflibercept group as compared with the sham group were eye pain (11.5% vs. 4.4%, respectively), increased intraocular pressure (8.7% vs. 5.9%, respectively), and conjunctival hemorrhage (8.7% vs. 4.4%, respectively) from baseline to week 24 and worsening of macular edema (35.1% vs. 10.5%, respectively), increased intraocular pressure (13.4% vs. 3.5%, respectively), and reduced visual acuity (11.3% vs. 1.8%, respectively) from weeks 24 to 52. All adverse events of intraocular pressure elevation were mild, except for 1 severe event that occurred in a sham patient before week 24. Ocular treatment-emergent serious adverse events (SAEs) are shown in Table 4. Most ocular SAEs were related to the disease state or injection procedure, and there were no clinically relevant differences between the treatment groups in terms of frequency or pattern of SAEs.

The incidence of nonocular TEAEs was similar in the sham and intravitreal aflibercept groups from baseline to week 24 (54.4% and 45.2%, respectively) and from weeks 24 to 52 (50.9% vs. 51.5%, respectively). Nasopharyngitis was the most commonly reported nonocular TEAE in both the sham and intravitreal aflibercept groups from baseline to week 24 (8.8% vs. 7.7%, respectively) and from weeks 24 to 52 (19.3% vs. 9.3%, respectively). Nonocular SAEs occurred in a small group of patients with a similar frequency in both the sham and intravitreal aflibercept groups from

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Table 2. Change from Baseline to Weeks 24 and 52 in the National Eye Institute 25-Item Visual Function Questionnaire Score

	Baseline to Week 24*				Baseline to Week 52†			
	Mean Change				Mean Change			
	Sham	Intravitreal Aflibercept Injection Monthly from Baseline to Week 24	Difference in Least Square Mean Change (95% Confidence Interval)	P Value	Sham	Intravitreal Aflibercept Injection Monthly from Baseline to Week 24 Plus PRN Treatment from Week 24 to 52	Difference in Least Square Mean Change (95% Confidence Interval)	P Value
Total score	3.5	7.5	4.2 (1.7–6.8)	0.0013	4.5	7.8	3.6 (1.1–6.0)	0.0049
Distance activities subscore	2.4	6.3	3.5 (−0.3 to 7.2)	0.0689	3.9	8.4	4.2 (0.4–7.9)	0.0283
Near activities subscore	1.6	10.4	8.6 (4.0–13.2)	0.0003	5.0	12.2	6.9 (3.1–10.8)	0.0005
Dependency subscore	2.4	3.7	2.1 (−1.6 to 5.8)	0.2552	3.1	3.8	1.6 (−1.7 to 4.8)	0.3423

PRN = pro re nata (as needed).
*n = 65 for sham and n = 96 for intravitreal aflibercept injection monthly from baseline to week 24.
†n = 67 for sham and n = 97 for intravitreal aflibercept injection monthly from baseline to week 24 plus PRN treatment from week 24 to 52 (except for the total score, which was n = 98).

baseline to week 24 (7.4% and 5.8%, respectively) and from weeks 24 to 52 (8.8% and 6.2%, respectively). None of the nonocular SAEs were reported for more than 1 patient from baseline to week 24. During weeks 24 to 52, nonocular SAEs reported for more than 1 patient were pneumonia (1 patient in each treatment group) and syncope (2 patients in the sham group and 1 patient in the aflibercept group). No adverse event was adjudicated as an Anti-Platelet Trialists' Collaboration-defined arterial thromboembolic event during the course of study. There were no deaths during the 52 weeks of this study.

Discussion

The findings of the current study demonstrate that the improvements in BCVA and CRT achieved with monthly intravitreal aflibercept injections in the first 24 weeks of treatment largely were maintained during the PRN (as-needed) phase of study, with monthly monitoring and a mean of 2.5 injections from weeks 24 to 52. Of note, there was also a marked improvement in BCVA with aflibercept in a subgroup of patients with nonperfused retinas at baseline, in contrast to a particularly poor response in the sham group. The visual improvements with aflibercept enhanced vision-related quality of life, particularly in near visual activities. In this study, aflibercept generally was well tolerated, and the most common adverse events were those typically associated with intravitreal injections or the underlying disease. The increase in macular edema seen in aflibercept patients during the PRN dosing phase suggests that some patients would have benefited from more regular dosing, rather than being treated in response to the recurrence of disease.

The sister study of GALILEO, the COPERNICUS study, demonstrated comparable improvements in BCVA and CRT with intravitreal aflibercept injections.[5,7] However, the sham groups in the 2 studies were not comparable during weeks 24 to 52 because, in the COPERNICUS study, sham patients received aflibercept PRN starting from week 24, whereas in the GALILEO study, sham patients continued to receive sham treatments through week 52. In the COPERNICUS study, patients receiving sham plus IVT-AFL PRN

Table 3. Distribution of Pro Re Nata Injections during Weeks 24 through 52 and Best-Corrected Visual Acuity Gains at Week 52 in Patients Treated with Intravitreal Aflibercept Injection Every 4 Weeks from Baseline to Week 24 and Pro Re Nata from Weeks 24 to 52

No. of Pro Re Nata Injections	Intravitreal Aflibercept Patients, n (%; n = 91*)	Change (Standard Deviation) from Baseline in Best-Corrected Visual Acuity at Week 52,† No. of Letters
0	13 (14.3)	19.8 (11.4)‡
1	12 (13.2)	
2	18 (19.8)	21.1 (12.8)§
3	21 (23.1)	
4	17 (18.7)	13.1 (13.5)‖
5	3 (3.3)	
6	7 (7.7)	

BCVA = best-corrected visual acuity; SD = standard deviation.
*Patients completing week 52.
†Because of the small number of patients in each injection category, BCVA gains at week 52 were shown for patients who received 0 to 1, 2 to 3, and 4 to 6 injections. The mean BCVA ± SD at baseline was 58.2±15.5 letters, 49.4±15.9 letters, and 55.4±15.0 letters for patients who received 0 to 1, 2 to 3, and 4 to 6 injections, respectively.
‡For both 0 and 1 injection categories.
§For both 2 and 3 injections categories.
‖For 4 to 6 injections categories.

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Table 4. Ocular Treatment-Emergent Serious Adverse Events in the Study Eye Occurring from Baseline to Week 24 and Weeks 24 to 52

	Baseline to Week 24*		Week 24 to Week 52†	
Serious Adverse Event	*Sham* ($n = 68$)	*Intravitreal Aflibercept Injection Monthly from Baseline to Week 24* ($n = 104$)	*Sham* ($n = 57$)	*Intravitreal Aflibercept Injection Monthly from Baseline to Week 24 and Pro Re Nata Treatment from Weeks 24 to 52* ($n = 97$)
Total no. of patients with ≥1 SAE, n (%)	5 (7.4)	2 (1.9)	2 (3.5)	8 (8.2)
Glaucoma	1 (1.5)	0 (0)	1 (1.8)	0 (0)
Iris neovascularization	0 (0)	1 (1.0)	0 (0)	0 (0)
Macular edema	2 (2.9)	0 (0)	0 (0)	4 (4.1)
Reduced visual acuity	1 (1.5)	0 (0)	0 (0)	1 (1.0)
Vitreous detachment	0 (0)	1 (1.0)	0 (0)	0 (0)
Vitreous hemorrhage	1 (1.5)	0 (0)	1 (1.8)	1 (1.0)
Macular fibrosis	0 (0)	0 (0)	0 (0)	1 (1.0)
Macular ischemia	0 (0)	0 (0)	0 (0)	1 (1.0)
Retinal detachment	0 (0)	0 (0)	0 (0)	1 (1.0)
Retinal vein occlusion	0 (0)	0 (0)	0 (0)	1 (1.0)

SAE = treatment-emergent serious adverse event.
*Safety analysis set.
†Week 24 completers within safety analysis set.

did not achieve visual and anatomic improvements as robustly as those receiving aflibercept from the inclusion at week 52, suggesting that patients with macular edema secondary to CRVO may benefit from initiating treatment early with aflibercept.[7]

Treatment of CRVO with monthly intravitreal injections for 6 months followed by monthly monitoring and PRN injections for an additional 6 months has been studied for the Ranibizumab for the Treatment of Macular Edema after Central Retinal Vein OcclUsIon Study: Evaluation of Efficacy and Safety (CRUISE) trial.[8] Visual and anatomic outcomes reported from the CRUISE study are comparable with those from the COPERNICUS and GALILEO studies, with gains achieved during the fixed monthly dosing phase largely maintained under PRN dosing with monthly monitoring.[8,12] However, it is suggestive that the steeper decline in visual acuity between months 6 and 7 with 0.5 mg ranibizumab in the CRUISE study, compared with the smaller decline seen with aflibercept during the same time period in the GALILEO study, is reflective of a longer duration of effect with aflibercept.

The GALILEO results at week 52 corroborate the robust effect on visual and anatomic measures seen at week 24 in patients with macular edema secondary to CRVO, after 24 weeks of fixed monthly dosing with aflibercept. Originally, the PRN dosing regimen was introduced to investigate the feasibility of extending the treatment interval after the initial monthly aflibercept dosing phase. It has been demonstrated that the PRN dosing regimen largely maintained the improvements seen at week 24 with monthly monitoring. During the PRN dosing phase, an average of 2.5 injections was given in the IVT-AFL 2Q4 + PRN group, which approximates the 3 injections that would have been administered using a bimonthly dosing regimen, as has been established for aflibercept in wet AMD patients.[11] From a practical perspective, the advantage of PRN dosing therefore is questionable: Although PRN dosing may lead to fewer injections than a fixed monthly regimen, it comes with the requirement of monthly visits. Therefore, a good alternative option would be flexibly adjusting the treatment interval using a treat-and-extend algorithm. This may help to preserve visual and anatomic gains better over PRN dosing as well as to reduce the challenges and cost of monthly monitoring.

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Footnotes and Financial Disclosures

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Targeted Therapy for Metastatic Colorectal Cancer: Role of Aflibercept

Edith P. Mitchell

Abstract

Worldwide, colorectal cancer (CRC) is the third most commonly diagnosed cancer in male individuals and the second most commonly diagnosed cancer in female individuals. Survival outcomes are less than optimal for patients with metastatic disease, with a 5-year survival in the 5% to 8% range. The development of new chemotherapeutic agents and effective combination regimens for metastatic colorectal cancer (mCRC) has increased median overall survival (OS) to the 24- to 28-month range. Because of the recognition that vascular endothelial growth factors (VEGFs) and their receptors are primary regulators of physiologic and pathologic angiogenesis and lymphangiogenesis, leading to neovascularization and tumor growth, the targeting of the angiogenic pathway has become a focus of key therapeutic strategies in mCRC. Therapeutic regimens that include bevacizumab, an inhibitor of VEGF-A, in combination with cytotoxic chemotherapy, have resulted in improved response rate (RR) and survival in mCRC. However, the effects of VEGF-A inhibition are often temporary, with resistance and disease progression developing in most patients. Proposed models include intrinsic and adaptive resistance, mediated by factors other than VEGF-A. Aflibercept (known as ziv-aflibercept in the United States; Zaltrap®, Regeneron Pharmaceuticals; sanofi-aventis), a novel recombinant fusion protein, is an angiogenic factor trap that blocks the binding of VEGF-A, VEGF-B, and placental growth factor. Phase I/II clinical trials have demonstrated effective activity in mCRC, with acceptable safety and tolerability. A recent phase III randomized double-blind trial in patients previously treated with oxaliplatin reported significant improvement in OS, progression-free survival (PFS), and RR with aflibercept compared with placebo when administered in combination with irinotecan and fluorouracil. Adverse events were consistent with anti-VEGF therapy. Thus aflibercept represents a potential new treatment option for patients with mCRC.

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in male individuals and the second most commonly diagnosed cancer in female individuals, with more than 1.2 million new cases worldwide; in 2008 it was the cause of 608,700 deaths.[1] Although CRC incidence and death rates have shown decreases in the United States, it is anticipated that 143,460 new cases of CRC will be diagnosed and approximately 51,690 Americans will die of the disease, accounting for approximately 9% of cancer deaths.[2] The lifetime incidence of CRC in patients at average risk is approximately 5%, with 90% of cases occurring after age 50 years.[3] In the United States, CRC incidence declined roughly 2% to 3% per year between 1992 and 2008.[4] Information from the Surveillance Epidemiology and End Results (SEER) database suggests that incidence rates are increasing in the 40- to 44-year age group.[5] Furthermore, CRC incidence in the United States is 25% higher in men than in women and is almost 20% higher in African Americans than in whites.[2] For most patients with metastatic disease, treatment remains palliative, and survival outcomes are less than optimal. Despite advances in management, metastatic disease is associated with poor 5-year survival, with a rate of approximately 10%.[6] It is estimated that 20% of patients with CRC have metastatic disease at the time of diagnosis, whereas many others will experience metastases during the course of the disease.[7] SEER data suggest that 64% of all patients, including all stages of disease, who are treated for CRC survive 5 years.[3] A recent clinical trial achieved an overall survival (OS) rate of 72.9% at 6 years in patients with stage III disease who were treated with oxaliplatin-based postoperative adjuvant chemotherapy.[8]

In the early 1970s, Folkman et al reported the importance of novel growth and remodeling of blood vessels in the growth and proliferation of tumors.[9] This work led to the development of biological

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agents that target angiogenesis. The elucidation of the role of vascular endothelial growth factors (VEGFs) in tumorigenesis contributed to important advances for the treatment of patients with a variety of solid tumors, including metastatic CRC (mCRC). During the past 2 decades, the addition of bevacizumab combined with cytotoxic chemotherapy has resulted in unprecedented advances in the treatment of mCRC, with improved response, progression-free survival (PFS), and OS.[10,11] This review will discuss the role of angiogenesis in mCRC, the principles of resistance to antiangiogenic therapy, and the development of aflibercept (known as ziv-aflibercept in the United States; Zaltrap®, Regeneron Pharmaceuticals; sanofi-aventis) as a novel antiangiogenic agent for mCRC.

Angiogenesis

Angiogenesis is a pivotal process for growth, invasion, and metastasis in many solid tumors.[9,12] Although physiologic angiogenesis is a highly regulated process,[13,14] pathologic angiogenesis manifests as an abnormal increase in proliferating endothelial cells and as structural and functional abnormalities of tumor vasculature.[15,16] These abnormalities include loss of normal vascular hierarchy, development of an irregular and leaky endothelial layer, compression of vessels, abnormal blood flow, loss of functional lymphatic vessels, increased interstitial pressure, and development of hypoxia and acidosis in the tumor microenvironment. Other abnormalities involve the pericytes and basement membrane of the tumor vasculature, and endothelial cells in tumor vessels show altered gene expression.[16,17]

Angiogenesis is controlled by a complex signaling network that involves multiple interacting proangiogenic and antiangiogenic signals, including VEGF, angiopoietins, Notch, and integrins.[18,19] The variety of pathways involved in angiogenesis offers numerous possible therapeutic targets. The VEGF family has been widely implicated as a key regulator of tumor angiogenesis[20,21] and is composed of 5 growth factors: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF).[15,21,22] These growth factors differentially bind and activate 3 cell surface tyrosine kinase receptors, VEGFR-1, VEGFR-2, and VEGFR-3, the activities of which can be enhanced by coreceptors neuropilin receptor (NRP)-1 and NRP-2.[23-25]

Mechanism of Angiogenesis

VEGF-A was the first member of the VEGF family to be identified and is recognized as the most potent inducer and positive regulator of the normal and pathologic angiogenic cascade.[26,27] It regulates blood vessel proliferation and vascular permeability, and its expression is associated with poor prognosis in a variety of human cancers.[28] The biological effects of VEGF-A include endothelial cell proliferation, survival, migration, invasion, chemotaxis of bone marrow progenitors, vascular permeability, and vasodilation, which are mediated by its binding and activation of receptor tyrosine kinases VEGFR-1 and VEGFR-2.[15] Although VEGF-A binds VEGFR-1 with approximately 10 times higher affinity than VEGFR-2, the higher kinase activity of VEGFR-2 makes it the most important effector of VEGF-A signaling.[19,20,26,29-32] Although VEGF-A is the best-characterized member of the VEGF family, experimental and clinical evidence indicates that other VEGFs, such as VEGF-B and PlGF, play an important role in tumor biological processes and pathologic angiogenesis.[22,33]

VEGF-B shares close structural homology with VEGF-A; although these 2 factors are coexpressed in many tissues, VEGF-B is more broadly expressed in skeletal muscle and the pancreas.[34] VEGF-B binds VEGFR-1 and NRP1 and although still under investigation, it could play a role in tumorigenesis and angiogenesis.[35] This is postulated because both VEGF-B and VEGFR-1 are upregulated in a number of different tumor types—in some cases, correlating with poor prognosis, metastasis, and relapse.[22] VEGF-B has been shown to have pleiotropic effects on vascular cell adhesion,[33,36] and although it seems to be dispensable for the growth of blood vessels, VEGF-B may play a role in the survival of preexisting blood vessels under pathologic conditions.[36]

PlGF exists as at least 4 different isoforms originated by alternative splicing: PlGF-1, PlGF-2, PlGF-3, and PlGF-4.[37] Similar to VEGF-B, PlGF is also homologous to VEGF-A and binds VEGFR-1.[22,38] Levels of both PlGF-1 and PlGF-2 have been shown to be elevated in human colorectal tumors.[39] There is also evidence that PlGF potentiates the response to VEGF-A by signaling through VEGFR-1,[40] and this signaling stimulates the recruitment of bone marrow–derived macrophages to the tumor site, where they release angiogenic factors.[41]

Because of the central role of the VEGF family in angiogenesis, and the increased VEGF expression in many tumor types, this family of growth factors has become an important therapeutic target. Anti-VEGF therapies currently available for the treatment of mCRC include the monoclonal antibody bevacizumab,[42] which binds VEGF-A, as well as the monoclonal antibodies cetuximab[43] and panitumumab,[44] which indirectly inhibit angiogenesis by targeting the EGF receptor. Nonetheless, there has been a lack of patient response to anti-VEGF therapies, and the clinical benefits observed are short-lived. Tumor regrowth and disease progression often occur after an initial response, indicating that cancer cells are able to functionally evade therapeutic inhibition of angiogenesis.[45-47]

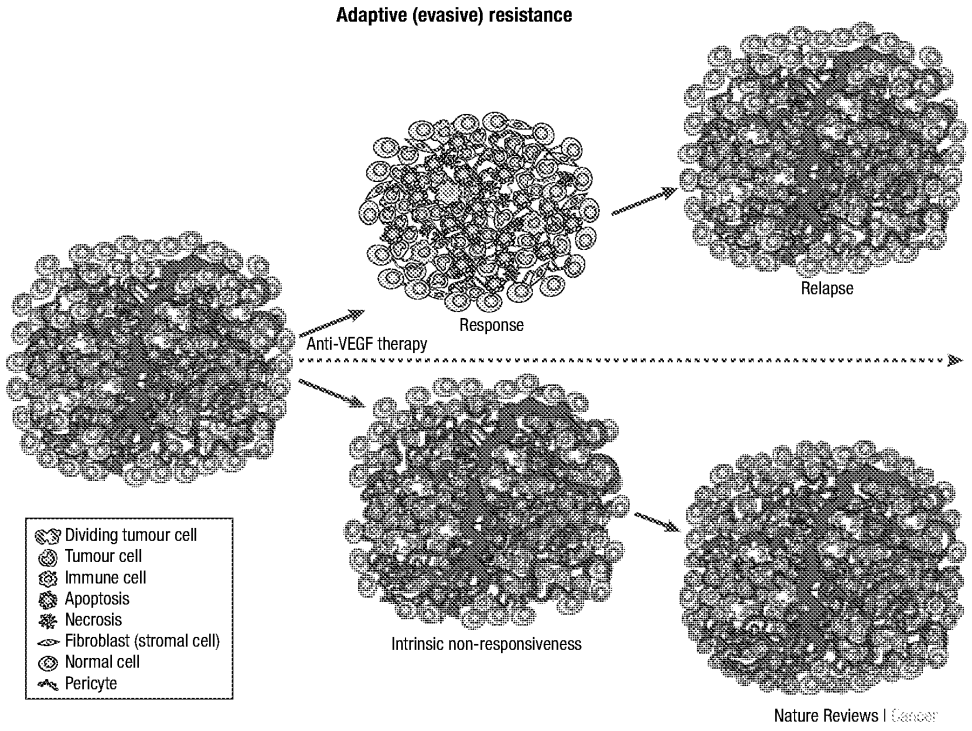
Mechanisms of Resistance To Antiangiogenic Therapy

Despite initial success, resistance to antiangiogenic therapy eventually develops, thus limiting survival benefits. Because multiple pathways contribute to angiogenesis, it is possible that 1 or more may contribute to the development of resistance. Possible specific mechanisms of resistance include upregulation of VEGF receptors, the fibroblast growth factor signaling pathway, interleukin-12, hepatocyte growth factor, increased pericyte coverage of tumor blood vessels to support vasculature, and recruitment of alternative angiogenic factors and pathways such as VEGF-C or VEGF-D.[48,49] Two broad mechanisms, adaptive and intrinsic resistance, have been proposed to explain the lack of effectiveness of anti-VEGF therapy (Figure 1). Adaptive resistance develops after an initial positive antiproliferative response, whereas intrinsic resistance exists in tumors before treatment.[50] The key mechanisms include (1) revascularization after upregulation of alternative proangiogenic pathways, (2) protection of the tumor vasculature either by the recruitment of vascular progenitor cells and proangiogenic monocytes from the bone marrow to the peritumoral area or by increasing protective pericyte coverage, (3) enhanced capability of tumor cells to migrate and invade local tissue to coopt normal vasculature, and (4) increased metastatic spread and tumor cell growth in lymph nodes and distant organs. Intrinsic re-

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Figure 1. Mechanisms of Resistance to Antiangiogenic Therapy[55]



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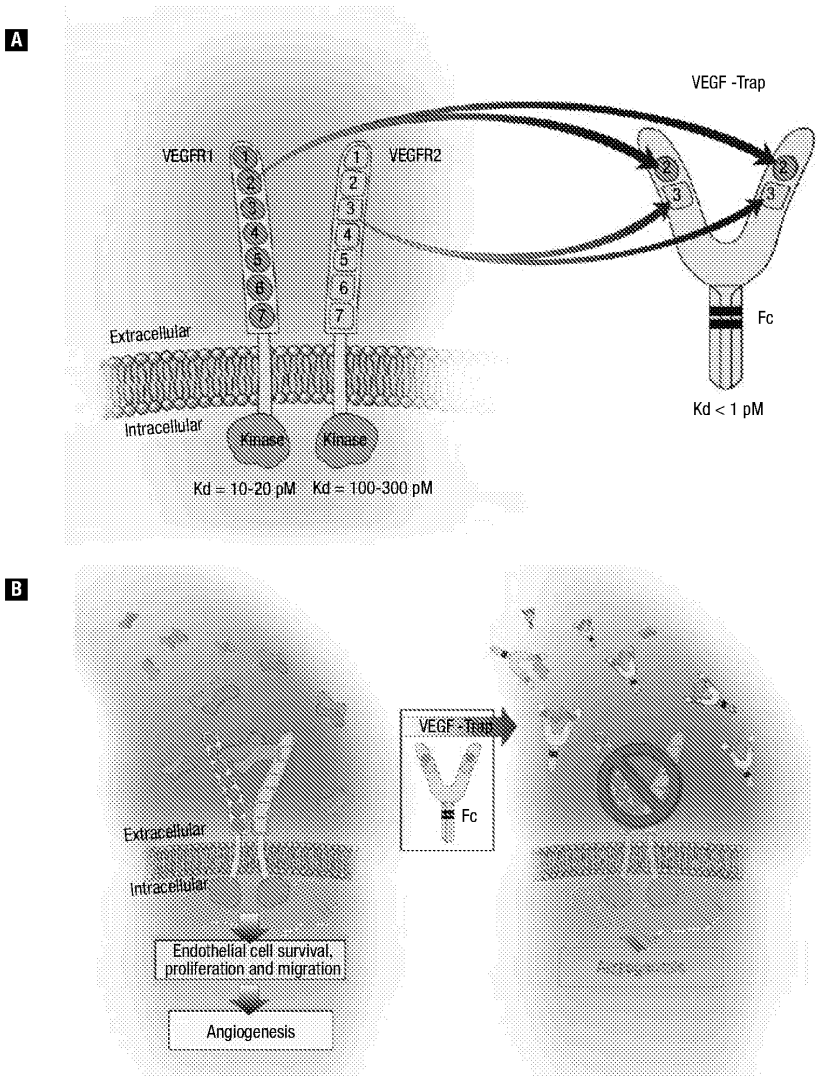
sistance is thought to involve many of these same mechanisms. In addition to rapid adaptation, intrinsic resistance may result from (1) multiplicity of proangiogenic signals, (2) vascular protection mediated by inflammatory cell or hypovascular tumor microenvironment characterized by indifference toward angiogenesis inhibitors, or (3) invasive cooption of normal vessels in the absence of neoangiogenesis (Figure 1).[50] It has been suggested that future cancer therapeutic strategies will integrate inhibitors of angiogenesis with drugs targeting resistance mechanisms to provide more enduring efficacy.[49,50] Two recent studies in mCRC have shown changes in circulating angiogenic biomarkers after the inhibition of VEGF-A with bevacizumab. The addition of bevacizumab to 5-fluorouracil (5-FU), leucovorin, irinotecan, and oxaliplatin (FOLFOXIRI) resulted in a prolonged and significant reduction in levels of VEGF-A through the time of disease progression. However, the soluble VEGFR-2 and PlGF levels increased from baseline during treatment, potentially contributing to disease progression.[51] This is consistent with observations from other studies that demonstrate that PlGF promotes tumor angiogenesis and tumor growth and may also contribute to tumor "escape" by providing sufficient angiogenic signals when VEGF-A is blocked.[22,52,53] In a second study, patients with mCRC (n = 43) with no previous chemotherapy for metastatic disease were treated with 5-FU, leucovorin, and irinotecan (FOLFIRI) in combination with bevacizumab.[54] There was no association between baseline levels of VEGF or VEGFR-2 and differences in PFS or OS.[54] However, compared with baseline levels, initial treatment resulted in an increase in PlGF ($P = .01$), soluble VEGFR-2 ($P = .03$), and eotaxin ($P = .01$), whereas hepatocyte growth factor ($P = .046$), basic fibroblast growth factor ($P = .047$), PlGF ($P < .001$), stromal derived factor-1 ($P = .038$), and macrophage chemoattractant protein-3 ($P < .001$) were elevated before disease progression.[54] At the time of progression, the PlGF level declined from its peak but remained greater than baseline ($P < .01$), whereas the VEGFR-2 ($P < .001$) and soluble VEGFR-2 ($P = .005$) levels declined to less than baseline.[54] It was suggested that increases in the levels of uninhibited angiogenic factors, such as PlGF, are compensatory mechanisms to stimulate new vessel growth in preparation for disease progression.[54]

The development of resistance by some tumor types to long-term antiangiogenic therapy has contributed to the search and development of treatment options intended to address the proposed mechanisms of resistance to antiangiogenic therapy. A review of many of these strategies,

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Figure 2 Structure and Mechanism of Action of Aflibercept.[55] (A) Aflibercept Consists of a Portion of VEGFR-1 and Domain 3 of VEGFR-2 Fused to the Fc Portion of IgG1. (B) Aflibercept Binds and Blocks Its Substrates and Prevents It from Activating the Native Receptors



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such as regorafenib, cediranib, AMG 386, and RO5323441 (TB 403) can be found in a recent review by Tejpar et al.[48]

Aflibercept

Aflibercept, a soluble recombinant fusion protein, is a multiple angiogenic factor trap rationally designed to block the angiogenesis network by not only binding VEGF-A but also uniquely targeting VEGF-B and PlGF.[53-57] Aflibercept was developed by fusing sections of the second immunoglobulin (Ig) domain of VEGFR-1 and the third Ig domain of VEGFR-2 to the F_c portion of human IgG1 (Figure 2).[55,58] Aflibercept has now been approved by the US Food and Drug Administration, with the US name of ziv-aflibercept, for

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Table 1 Pharmacokinetics of Total Aflibercept[55]

Parameter	Dose Level (mg/kg)						
	0.3	1.0	2.0	3.0	4.0	5.0	7.0
C_{max} (μg/mL)	4.00 ± 9%	17.9 ± 31%	34.5 ± 11%	46.7 ± 30%	97.4 ± 43%	86.8 ± 34%	159 ± 21%
C_{last} (μg/mL)	0.147 ± 38%	0.659 ± 116%	2.36 ± 71%	4.06 ± 63%	11.0 ± 51%	9.63 ± 28%	14.4 ± 55%
AUC (day·μg/mL)	9.34 ± 15%	50.9 ± 54%	125 ± 35%	226 ± 34%	293 ± 15%	428 ± 64%	605 ± 46%
$t_{1/2}$ (day)	1.70 ± 21%	2.58 ± 50%	3.76 ± 42%	6.18 ± 38%	5.51 ± 18%	7.43 ± 38%	5.14 ± 37%
V_{ss} (L)	4.51 ± 29%	5.88 ± 22%	5.58 ± 21%	7.74 ± 33%	7.88 ± 38%	9.89 ± 31%	6.12 ± 29%
Cl (L/d)	1.95 ± 42%	1.87 ± 51%	1.13 ± 31%	1.14 ± 48%	1.10 ± 38%	1.27 ± 65%	0.915 ± 39%

Abbreviations: AUC = area under the curve extrapolated to infinity; Cl = total body clearance; C_{last} = last measurable (non-zero) plasma concentration; C_{max} = maximum observed plasma concentration; $t_{1/2}$ = apparent terminal half-life; V_{ss} = volume of distribution at steady state.
Values are ± coefficient of variation (%).
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the use in combination with FOLFIRI in the treatment of mCRC that is resistant to or has progressed following an oxaliplatin-containing regimen.[59]

Pharmacokinetics and Pharmacodynamics

In animals, aflibercept has been found to have negligible binding to extracellular tissues.[55,60] Aflibercept forms a stable and inert 1:1 complex with VEGF, which appears in the circulation at maximal levels within 24 to 48 hours of treatment.[60] Clearance occurs via F_c receptor- or pinocytosis-mediated pathways that result in proteolysis.[61] With a K_d of 0.49 pM, aflibercept binds VEGF-A with affinities that are 19- and 181-fold higher than the native VEGF receptors fused to F_c (VEGFR-1 and VEGFR-2, respectively).[62] Aflibercept also exhibits strong affinity for PlGF-2, with a K_d of approximately 45 pM.[55]

The pharmacokinetics of subcutaneous aflibercept were characterized in a phase I trial by Tew et al. Patients (n = 38) received aflibercept subcutaneously at doses of 25, 50, 100, 200, 400, or 800 μg/kg weekly or 800 μg/kg twice weekly for 10 weeks, with continuation until disease progression.[56] Dose-dependent increases of free aflibercept were observed peaking within 3 days after the dose. Clearance of free aflibercept was relatively rapid, with an elimination half-life ($t_{½}$) up to 3 days compared with approximately 18 days for bound aflibercept. Formation of the VEGF/aflibercept complex appeared to saturate at approximately 800 μg/kg weekly, suggesting that much of the VEGF produced by the patient is being captured at this dose.[56]

When administered intravenously in doses ranging from 0.3 to 7.0 mg/kg every 2 weeks (n = 47), the mean maximum plasma concentration of free aflibercept increased with doses ranging from 4 to 159 μg/mL, whereas the exposure (area under the curve [AUC]) increased more than the dose proportionally in the 0.3- to 2.0-mg/kg dose range (Table 1).[55] The apparent $t_{½}$ also increased with doses ranging from 1.7 to 5.1 days. Clearance decreased at low doses but was stable at 2.0 to 7.0 mg/kg, and the concentration of free aflibercept remained greater than bound aflibercept throughout the dosing intervals at doses of ≥ 2.0 mg/kg, indicating binding saturation of endogenous VEGF. Bound aflibercept concentrations increased after cycle 1 and up to 3 weeks after the first dose, which indicates that steady state was not achieved during this time.[63]

Aflibercept Activity In Animal and Human Models

Subcutaneous single-agent aflibercept was effective at inhibiting tumor growth in mice models of mouse B16F10.9 melanoma, human A673 rhabdomyosarcoma, and rat C6 glioma, and almost completely blocked tumor-associated angiogenesis at the highest dose of 25 mg/kg (Figure 3A).[55] Aflibercept 2.5 mg/kg, comparable to the dose that inhibited tumor growth, was less effective at completely blocking tumor-associated angiogenesis, with small pockets of tumor-associated vessels observed (Figure 3B).[55] Similar dose-dependent effects have been observed in a xenograft model of neuroblastoma, whereas high doses of aflibercept led to greater regression of coopted vascular structures, which occurs during the initial phase of tumor growth.[64] Single-agent aflibercept also diminished tumor vasculature and volume in lung tumors in mice.[65] Furthermore, preexisting lung micrometastases markedly decreased in both size and cell number compared with controls, with evidence of apoptosis after 1 dose of aflibercept.[65]

The effect of aflibercept on activated signaling pathways in endothelial cells has been investigated in SCID mice bearing K1735 tumors or COLO 205 human colon cancer tumors.[66] Aflibercept significantly decreased tumor endothelial p-ERK, p-STAT3, and p-AKT expression ($P \leq .05$), with signs of antiangiogenesis.[66]

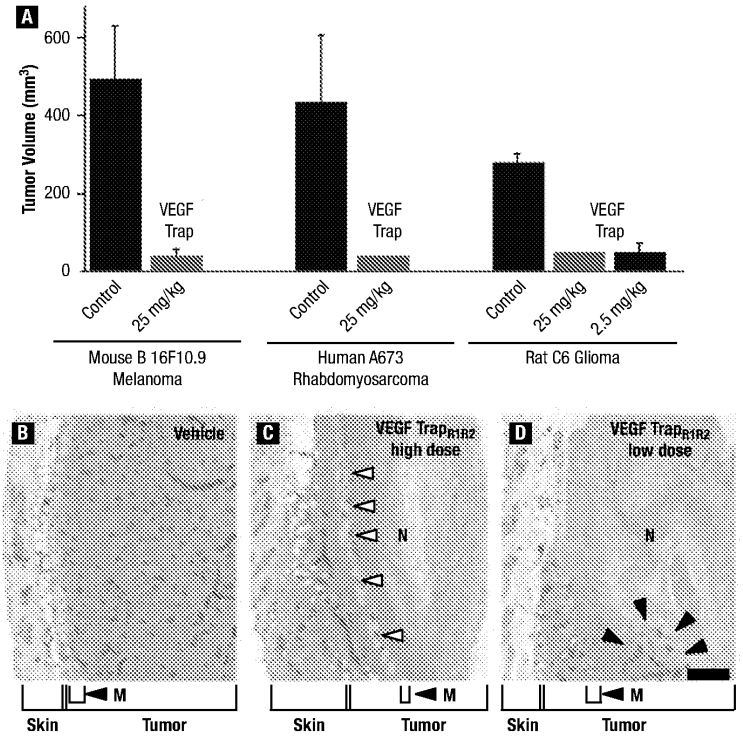
Aflibercept has been investigated alone and in combination with cytotoxic chemotherapy in several 3-arm investigations in mice. In a mammary adenocarcinoma study, aflibercept 40 mg/kg/dose twice weekly was as active as the highest nontoxic dose of 5-FU (90 mg/kg/dose), with the combination showing synergistic activity at all doses tested. Single-agent aflibercept and irinotecan were equally active in mice with advanced-stage human colon cancer, with the combination demonstrating synergy (Figure 4).[67] In BALB/c nude female mice with early-stage B16 melanoma, as well as gemcitabine in SCID mice with advanced human colon cancer, single-agent activity with aflibercept was comparable to docetaxel in additional studies. In both studies, the combination was more active than either agent alone.[68] Furthermore, these 4 studies presented little to no overlap in host toxicity with combination therapy.

The combination of aflibercept and docetaxel has shown activity in HT1080 tumors with vasculature that exhibits resistance to VEGF blockade. Although single-agent therapy exhibited only modest ef-

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Figure 4 Effect of Potent Soluble Antagonist (VEGF Trap) on Tumor Cell Growth and Vascularity.[69] Aflibercept dramatically Inhibits the Subcutaneous Growth and Vascularity of Established Tumors Cells Derived Tumors and Species. (A) Aflibercept Substantially Blocked the Growth of the Indicated Subcutaneously Implanted Tumors of the Indicated Species Twice Weekly for 2 Weeks (C6 and B16F10.9) or 3.5 Weeks (A673). Error Bars Represent Standard Error of Mean. * = Five Animals/Treatment Group. The Difference Between Control Tumor Volumes and Aflibercept Treated Tumor Volumes were Analyzed by Using Student *t* Tests and were Found to be Significant at the Following Levels: B16F10, $P \leq .01$; A673, $P \leq .05$; C6, $P \leq .0001$. (B–D) Histologic Analysis Reveals that Aflibercept can Effectively Block Blood Vessel Growth in these Implanted Tumors. Sections of C6 Tumors Stained with Antibodies to Platelet Endothelial Cell Adhesion Molecule Reveal that Vehicle Treated Animals had Large Tumors that were Highly Vascularized (B), whereas Animals Treated with Aflibercept 25 mg/Kg (C) had Tumors that were Largely Avascular with Large Areas of Necrosis (N). Viable Tumors Appeared to be Preordered Because of Retention of Supporting Host Vessels (White Arrowheads) Associated with Hypodermal Musculature (M) and Dermis. Treatment with Aflibercept 2.5 mg/kg Greatly Studied Tumor Growth (C) and Resulted in Large Necrotic Regions (N), Although Small Pockets of Viable Cells Occasionally Appeared (Black Arrowhead) (Bar = 100 μm.)



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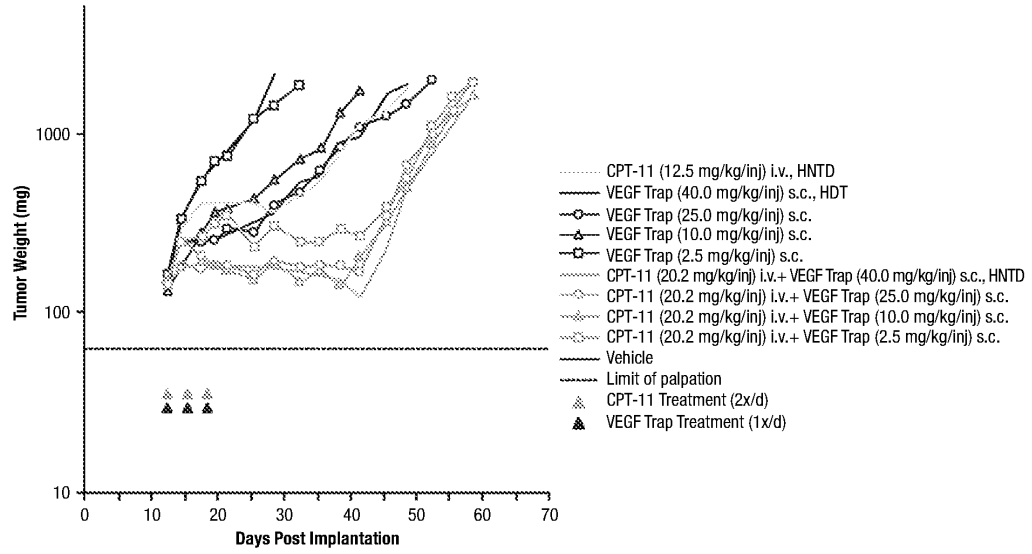
fects on tumor vessels, the combination of aflibercept and docetaxel resulted in pruned vessels with less branching, some vessels with punctuate endothelial cell staining, and an increase in terminal dUTP nick end labeling–positive endothelial cells.[69]

Compared with bevacizumab and doxorubicin, enhanced antitumor effects with aflibercept have been shown in vivo in human VEGF-expressing acute myeloid leukemia models, in which aflibercept treatment induced increased tumor ablation and areas of central necrosis surrounded by rims of proliferating leukemia cells. This suggests both direct and indirect effects with aflibercept in combination with doxorubicin, not simply the decreased tumor blood flow that was also observed.[70] Aflibercept treatment followed by doxorubicin showed progressive anthracycline accumulation in extramedullary acute myeloid leukemia sites and marrow, resulting in up to 2-fold higher doxorubicin concentrations after 24 hours. In contrast, mice not pretreated with aflibercept generally showed progressive drug clearance over time. Although the aflibercept mechanisms leading to variable doxorubicin levels in different

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Figure 4 Synergy Between Aflibercept (VEGF Trap) and Irinotecan in Colon Cancer Xenografts (colon HCT-116 in nude mice)[56]



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tissues are uncertain, there is the suggestion that inefficient drug delivery by leukemia-associated vasculature, which may mediate chemoresistance, may be positively influenced by aflibercept.[70]

In summary, preclinical studies in various mouse and human models have shown antiangiogenic activity with aflibercept alone or in combination with cytotoxic chemotherapy, causing vascular remodeling and tumor growth inhibition and regression. Furthermore, pretreatment with aflibercept increases tumor cell kill compared with single-agent cytotoxic chemotherapy by increasing cytotoxic drug exposure and possibly by other mechanisms.

Clinical Trials

The efficacy and safety of aflibercept alone or in combination with various chemotherapy regimens has been explored in several phase I[56,63,71-76] and phase II[77-87] trials in patients with advanced solid tumors or non-Hodgkin lymphoma. Solid tumors included breast, colorectal, endometrial, gastric, glioblastoma, lung, melanoma, ovarian, pancreas, sarcoma, thyroid, urothelial, and others. Aflibercept has also been investigated in a phase III trial involving patients with mCRC.

Phase I Trials

Aflibercept has been administered subcutaneously or intravenously in phase I clinical trials.[56,63,71-76] Three phase I clinical trials have investigated the safety, pharmacokinetics, and pharmacodynamics of aflibercept as a single agent every 2 weeks in patients with advanced solid tumors (Table 2). Lockhart et al enrolled 47 patients with refractory solid tumors at a starting dose of 0.3 mg/kg, which was 10-fold less than the dose from primate studies that produced no observed effect.[63] Patients who tolerated the first 2 doses of aflibercept were eligible to receive treatment at 7 dose levels on the long-term tolerability study. Three patients had an objective partial response (PR). The following dose-limiting toxicities occurred: grade 3 elevation in alanine aminotransferase levels at 1.0 mg/kg, grade 3 dyspnea and arthralgia at 2.0 mg/kg, and grade 3 hypertension at 4.0 mg/kg. The study was subsequently modified to allow for cohort expansion, provide more intensive blood pressure control before defining a dose-limiting toxicity, and allow for higher levels of proteinuria. The subsequent dose-limiting toxicities occurred at the 7.0-mg/kg dose level with 1 episode each of proteinuria and rectal ulceration. The most common antiangiogenic adverse events (AEs) were dysphonia, hypertension, and proteinuria. Other common, generally grade 1, AEs were fatigue, nausea, and vomiting. Maximal VEGF/aflibercept complex levels were reached at doses of 2 mg/kg and greater.

A second trial involved 38 patients with advanced solid tumors who had received a median of 5 previous chemotherapy regimens.[56] The initial cohort of 3 patients received subcutaneously administered aflibercept at 25 μg/kg to investigate the pharmacokinetics of aflibercept. Five additional patients were enrolled at either the maximum administered dose or the maximum tolerated dose (dose level at less

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Table 2. Results of Phase I, II, and III Clinical Trials of Aflibercept in Colorectal Cancer

Patient Population	Treatment	Results	Major Adverse Events (All Grades)	Major Findings	Reference
Phase I					
Refractory solid tumors (n = 47) including CRC (n = 7)	Aflibercept (0.3-7 mg/kg)	PR 6%; SD (> 1 y) 4% (CRC group not specified) DLT rectal ulceration and proteinuria (7 mg/kg) Maximum VEGF blockade at doses ≥ 2 mg/kg	Fatigue 64%, dysphonia 47%, HTN 38%, nausea 36%, constipation 32%, headache 32%, vomiting 28%, arthralgia 26%	Aflibercept was safely administered at doses that demonstrated antitumor activity	Lockhart et al[63]
Refractory solid tumors (n = 38) including CRC (n = 6)	Aflibercept (0.025-0.8 mg/kg)	SD (≥ 10 wk) 47% (CRC group not specified) Maximum VEGF blockade at 0.8 mg/kg	Proteinuria 37%, fatigue 32%, injection-site reaction 18%, nausea 17%, musculoskeletal discomfort 16%, anorexia 16%, HTN 13%, hoarseness 11%	Aflibercept was well tolerated and had manageable side effects	Tew et al[64]
Heavily pretreated advanced solid tumors (n = 54) including CRC (n = 9)	Aflibercept (2-9 mg/kg) + docetaxel	DLT at cycle 1 included neutropenic infection (2 mg/kg), grade 2 HTN and grade 3 dysphonia (7 mg/kg), grade 2 HTN (9 mg/kg) PR 9%, SD 69% (CRC group not specified)	Neutropenia 98%, fatigue 89%, hemorrhage 81%, anemia 81%, stomatitis 72%, dysphonia 64%, HTN 53%, musculoskeletal disorders 53%, alopecia 51%	Aflibercept 6 mg/kg with docetaxel 75 mg/m^2 is recommended for further investigation	Isambert et al[73]
Pretreated mCRC (n = 16)	Aflibercept (2 or 4 mg/kg) + irinotecan + 5-FU + leucovorin (fixed doses)	Response rate 8%, PFS 7.6 mo No DLT	Grade 3/4: neutropenia (75%), hypertension (25%)	Recommended dose of aflibercept is 4 mg/kg in combination with FOLFIRI	Yamazaki et al[65]
Heavily pretreated advanced solid tumors (n = 16) including CRC (n = 2)	Aflibercept (4-6 mg/kg) + docetaxel + cisplatin	DLT without G-CSF prophylaxis: 17% (4 mg/kg) and 14% (5 mg/kg) DLT with G-CSF: 0% CR 7%, PR 7%, SD 50% (CRC group not specified)	Grade 2: epistaxis 19%, proteinuria 17%, dysphonia 13% Grade 3/4: HTN 13%	Recommended dose of aflibercept is 6 mg/kg in combination with standard docetaxel + cisplatin	Freyer et al[74]
Heavily pretreated advanced solid tumors (n = 32) including CRC (n = 1)	Aflibercept (2-5 mg/kg) + oxaliplatin + leucovorin + 5-FU (FOLFOX)	No DLT during dose escalation Free/bound aflibercept ratio (trough concentration) 0.74-4.5 PR 16%, SD 31% In CRC: PR 100%	Fatigue 81%, nausea 72%, skin/subcutaneous tissue disorder 56%, anorexia 53%, diarrhea 53%, vomiting 53%, abdominal pain 50%, headache 50%, HTN 50%, musculoskeletal/connective tissue disorder 50%	Aflibercept 4 mg/kg with FOLFOX is recommended for further investigation	Limentani et al[71]
Advanced solid tumors (n = 38) including CRC (n = 23)	Aflibercept (2-6 mg/kg) + irinotecan + 5-FU + leucovorin (LV5-FU2)	PR 18%, SD 50% In CRC: PR 17%, SD 61% (> 12 mo 26%) DLTs were grade 3 proteinuria (4 mg/kg), grade 3 stomatitis and esophageal reflux (5 mg/kg), and febrile neutropenia and grade 3 stomatitis (6 mg/kg)	Diarrhea 92%, fatigue 92%, nausea 84%, stomatitis 82%, anemia 82%, neutropenia 76%, HTN 74%, dysphonia 74%, infections 71%, musculoskeletal disorder 68%, hemorrhage 68%, constipation 66%, vomiting 61%, anorexia 61%, headache 61%, dyspnea 55%, alopecia 50%, abdominal pain 42%, thrombocytopenia 42%	Aflibercept 4 mg/kg with LV5-FU2 is recommended for further investigation	Rixe et al[72]
Advanced solid tumors (n = 27) including CRC (n = 19)	Aflibercept (4 mg/kg) + irinotecan + 5-FU + leucovorin (I-LV5-FU2)	PR 4%, SD 78% In CRC: PR 5%	Grade 2: HTN 26%, dysphonia 11%, epistaxis 4%, proteinuria 4% Grade 3/4: HTN 15%	Aflibercept 4 mg/kg with I-LV5-FU2 is recommended for further investigation	Verslype et al[75]
Phase II					
Previously treated mCRC (n = 51)	Aflibercept (4 mg/kg)	Bevacizumab-naive: RR 29%; 4-mo PFS 29% Previous bevacizumab: RR 30%; 4-mo PFS 26% (CRC group not specified)	Fatigue 78%, HTN 55%, proteinuria 49%, headache 43%, voice alteration 31%, anorexia 24%, joint pain 18%	Single-agent aflibercept is active and well tolerated in previously treated mCRC	Tang et al[66]
Phase III					
mCRC previously treated with oxaliplatin-based therapy (n = 1226)	Aflibercept (4 mg/kg) + irinotecan + 5-FU (FOLFIRI) vs. placebo + irinotecan + 5-FU	Median OS: 13.5 vs. 12.06 mo (P = .0032) Median PFS: 6.9 vs. 4.67 mo (P = .00007) ORR: 19.8% vs. 11.1% (P = .0001)	Proteinuria (62% vs. 41%), HTN (41% vs. 11%), hemorrhage (38% vs. 19%), dysphonia (25% vs. 3%), headache (22% vs. 9%)	Addition of aflibercept to FOLFIRI resulted in significant improvement in OS and PFS and was unaffected by previous treatment with bevacizumab	Van Cutsem[67]

Abbreviations: CR = complete response; DLT = dose-limiting toxicity; 5-FU = 5-fluorouracil; FOLFOX = oxaliplatin + leucovorin + 5-FU; G-CSF = granulocyte colony-stimulating factor; HTN = hypertension; mCRC = metastatic colorectal cancer; ORR = overall response rate; OS = overall survival; PFS = progression-free survival; PR = partial response; RR = response rate; SD = stable disease; VEGF = vascular endothelial growth factor.

than which 2 of 6 patients exhibited a dose-limiting toxicity), whichever was lower. A duration of ≥ 10 weeks with stable disease was achieved in 18 patients (47%). The maximum administered dose was 800 μg/kg twice weekly, whereas the maximum tolerated dose was not reached. Dose-limiting toxicity was observed in 4 patients and resolved in 3 patients and included confusion and decreased oral

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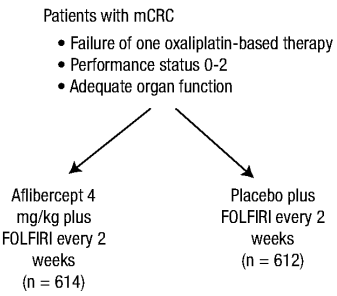
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Table 3 Adverse Events Reported in a 2-Stage Phase II Trial of Aflibercept in Patients with Refractory Colorectal Cancer[83]

Adverse Event	Most Frequent Adverse Events—Aflibercept (n = 51)	
	All Grades (%)	Grade 3/4 (%)
Fatigue	78.4	5.9
Decreased appetite	23.5	—
Joint pain	17.6	—
Anti–VEGF associated events		
Proteinuria	49.0	7.8
Hypertension	54.9	7.8
Dysphonia	31.4	—
Headache	43.1	5.9

Abbreviation: VEGF = vascular endothelial growth factor.

Figure 3 Study Design of Phase III VELOUR Trial[76]



intake, grade 3 proteinuria, and grade 3 leukopenia. Pulmonary embolism was diagnosed in the fourth patient after initiation of aflibercept, but was subsequently identified on baseline computed tomographic scan. Grade 3/4 AEs included hypertension, proteinuria, nausea, leukopenia, pulmonary embolism, and cerebral ischemia. Levels of the VEGF/aflibercept complex seen after the first dose did not increase appreciably between the 800 μg/kg once weekly and twice weekly dose levels.[56]

The aims of a third study were to document the dose-limiting toxicities occurring during the first treatment cycle and to establish the recommended dosage in patients with advanced solid tumors.[73] Patients with metastatic or unresectable disease or those for whom no standard conventional therapy existed received aflibercept 2 mg/kg with docetaxel 75 mg/m^2 on day 1 every 3 weeks until disease progression or unacceptable toxicity. The dose of aflibercept was escalated to 4, 5, 6, 7, and 9 mg/kg if no dose-limiting toxicity was observed in a cohort of 3 patients. If dose-limiting toxicity was observed in 1 patient, the cohort was expanded to 6 patients. The recommended dose was defined as the highest dose at which 2 of 3 to 6 patients experienced dose-limiting toxicity. The safety and activity of aflibercept were studied further in an expanded group after the recommended dose had been quantified. Seven patients experienced PR, and 18 patients experienced stable disease for more than 3 months. Dose-limiting toxicity during cycle 1 related to aflibercept was observed at the 7 and 9 mg/kg dose levels; consequently, 6 mg/kg was determined to be the recommended dose for aflibercept. All patients experienced 1 or more AEs. Antiangiogenic AEs included epistaxis, proteinuria, dysphonia, and hypertension.

The efficacy, safety, dose-limiting toxicities, recommended dose, and pharmacokinetics of aflibercept in combination with fixed doses of FOLFIRI as second-line therapy have been investigated in 16 patients with mCRC.[76] Aflibercept was administered at dosages of 2 or 4 mg/kg every 2 weeks, with 3 to 6 patients to be recruited at each dose. An additional 10 patients were to be treated at the recommended dose, defined as the highest dose of aflibercept at which < 33% of all evaluable patients experienced dose-limiting toxicity during the first 2 cycles. A total of 16 patients (3 receiving doses of 2 mg/kg and 13 receiving doses of 4 mg/kg) received a total of 131 cycles with no dose-limiting toxicity observed. The most common grade 3/4 AEs was neutropenia, including 1 case of febrile neutropenia and hypertension.

As single-agent therapy or in combination with chemotherapy, these trials suggested that aflibercept 4 mg/kg every 2 weeks or 6 mg/kg every 3 weeks were optimal doses based on antitumor efficacy and acceptable safety. In addition, the fraction of free aflibercept was found to be greater than bound aflibercept at these doses, with no apparent impact from concomitant chemotherapy on aflibercept pharmacokinetics. As expected, hypertension and proteinuria were the most common aflibercept-related AEs.

Phase II Trials

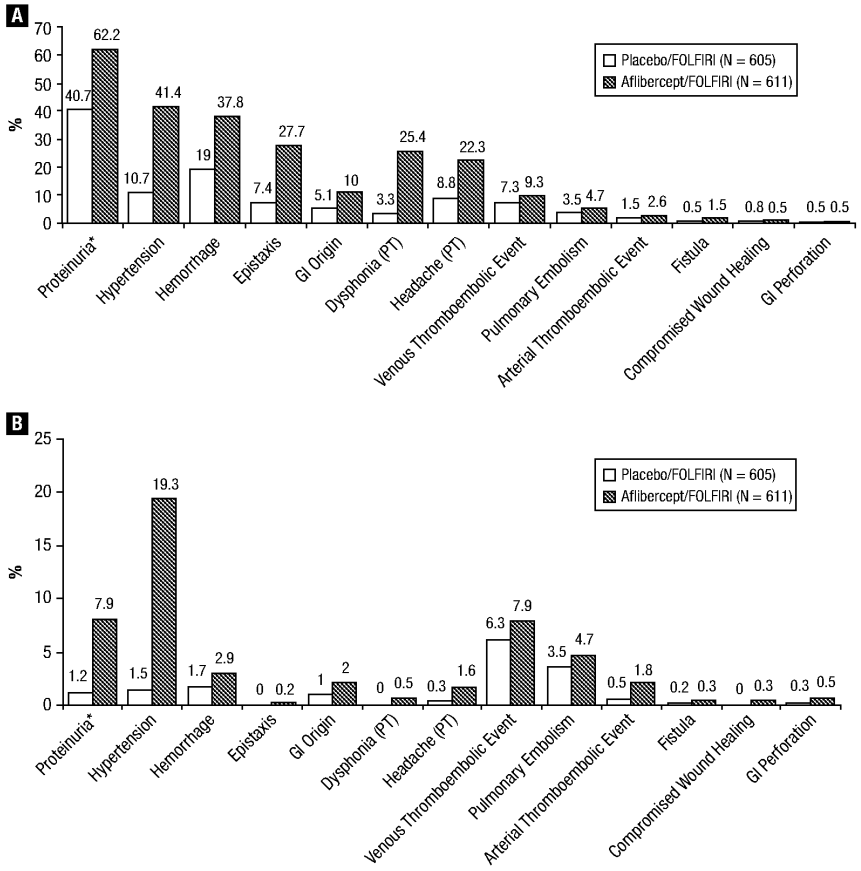
Phase II trials of aflibercept have investigated the efficacy and safety of single-agent aflibercept 4 mg/kg every 2 weeks given intravenously. Patients with gynecologic cancer,[77,80] lung cancer,[78,87] malignant ascites,[79,81] glioblastoma,[82] mCRC,[83] metastatic gynecologic soft tissue sarcomas,[88] melanoma,[85] or urothelial cancer[86] were generally heavily pretreated. Modest to moderate improvement in PFS was generally observed. For example, in patients with lung cancer (n = 98), the median PFS was 2.7 months and OS was 6.2 months, with 29% surviving at 12 months.[87] In patients with metastatic or locally advanced urothelial cancer previously treated with 1 platinum-containing regimen (n = 22), PFS was 2.79 months.[86] Disease progression was the most frequent reason for study discontinuation in these phase II trials. The most common aflibercept–related grade 3/4 AEs were those expected with antiangiogenic therapy, eg, hypertension, proteinuria, and fatigue, as well as headache and abdominal pain/perforation.

In heavily treated patients with mCRC, including those previously treated with bevacizumab, results from an open-label multicenter 2-stage phase II trial of patients with good performance status (PS) (Eastern Cooperative Oncology Group PS ≤ 2) (n = 51) demonstrated that aflibercept was well tolerated with modest activity (Table 3).[83] Twenty-seven patients had received previous bevacizumab therapy. After an average of 5.6 cycles of aflibercept 4 mg/kg intra-

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Figure 3 Adverse Events Reported in Phase III VELOUR Trial of Aflibercept in Patients with Metastatic Colorectal Cancer (mCRC).[57] (A) Percentage of All Grade Adverse Events, (B) Percentages of Grade 3/4 Adverse Events



Abbreviations: GI = gastrointestinal, PT = preferred term.

venously every 2 weeks, the median PFS was 3.4 months in the group previously treated with bevacizumab and 2.0 months in bevacizumab-naive patients. The most common aflibercept–related AEs were fatigue, hypertension, proteinuria, headache, voice alteration, anorexia, and joint pain (Table 2). Grade 3/4 treatment-related AEs occurring in more than 1 patient were hypertension (8%), proteinuria (8%), fatigue (6%), and headache (6%).

Aflibercept 6 mg/kg has also been investigated in combination with docetaxel 75 mg/m^2 given every 3 weeks in patients with recurrent epithelial ovarian, primary peritoneal, or fallopian tube carcinoma (n = 46).[80] After a median of 6 treatment cycles and a median follow-up of 24 months, an objective response (according to Response Evaluation Criteria in Solid Tumors) was achieved in 25/46 (54%) patients, 11 (24%) of whom achieved a complete response. The median PFS and OS were 6.4 and 26.6 months, respectively. AEs associated with aflibercept were grade 1/2 hypertension in 5 patients (11%) and grade 2 hypotension in 1 patient (2%).

Phase III Trial

Aflibercept in combination with FOLFIRI has also been investigated in a phase III trial (VELOUR; http://ClinicalTrial.gov ID NCT00561470) in patients with previously treated mCRC (n = 1226) who had adequate organ function and a PS of 0 to 2 and in whom a previous oxaliplatin-based regimen had failed.[57] Patients were a median of 61 years, 58.6% were men, 97.9% had a PS of 0 to 1, 56.4% had metastases in more than 1 organ, and 30.4% had received previous treatment with bevacizumab. Aflibercept 4 mg/kg on day 1 every 2 weeks (n = 614) or placebo (n = 612) was added to

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Table 4. Trials of Second-Line Treatment of Metastatic Colorectal Cancer With Aflibercept or Bevacizumab

Study	Patient Population	Treatment	Results	Grade ≥ 3 AEs
VELOUR (Van Cutsem et al[57])	mCRC previously treated with oxaliplatin-based therapy (n = 1226)	Aflibercept (4 mg/kg) + irinotecan + 5-FU (FOLFIRI) vs. placebo + irinotecan + 5-FU	Median OS: 13.5 vs. 12.06 mo (P = .0032) Median PFS: 6.9 vs. 4.67 mo (P = .0007) ORR: 19.8% vs. 11.1% (P = .0001)	Diarrhea (19.3% vs. 7.8%), asthenia (16.8% vs. 10.6%), stomatitis (13.8% vs. 5.0%), infections/infestations (12.3% vs. 6.9%), HTN (19.3% vs. 1.5%), GI/abdominal pains (5.4% vs. 3.3%), headache (1.6% vs. 0.3%), hand-foot syndrome (2.8% vs. 0.5%), thromboembolic event (9.6% vs. 6.7%), neutropenia (36.7% vs. 29.5%), thrombocytopenia (3.4% vs. 1.6%), proteinuria (7.8% vs. 1.2%)
E3200 (Giantonio et al[90])	mCRC previously treated with fluoropyrimidine and irinotecan (n = 829)	FOLFOX4 + bevacizumab 10 mg/kg vs. FOLFOX4 vs. bevacizumab 10 mg/kg	Median OS: 12.9 vs. 10.8 vs. 10.2 mo (P = .0011) PFS: 7.3 vs. 4.7 vs. 2.7 mo (P < .0001) ORR: 22.7% vs. 8.6% vs. 3.3% (P < .0001) (P values are for FOLFOX4 + bevacizumab vs. FOLFOX4)	HTN (6.2% vs. 1.8% vs. 7.3%), bleeding (3.4% vs. 0.4% vs. 2.1%), vomiting (10.1% vs. 3.2% vs. 4.7%), proteinuria (0.7% vs. 0% vs. 0%), neuropathy (16.3% vs. 9.2% vs. 0.8%), thromboembolism (3.4% vs. 2.5% vs. 0.4%), cardiac/cerebrovascular ischemia (0.9% vs. 0.4% vs. 0.4%)
TML (Arnold et al[91])	mCRC after first progression with oxaliplatin- or irinotecan-based chemotherapy plus bevacizumab (n = 820)	Irinotecan- or oxaliplatin-based chemotherapy + bevacizumab 2.5 mg/kg vs. irinotecan- or oxaliplatin-based chemotherapy	Median OS: 11.2 vs. 9.8 mo (P = .0062) Median PFS: 5.7 vs. 4.1 mo (P < .0001) ORR: 5.4% vs. 3.9% (P = .3113)	Neutropenia (16% vs. 13%), diarrhea (10% vs. 8%), leukopenia (4% vs. 3%), vomiting (4% vs. 3%), abdominal pain (4% vs. 3%), asthenia (6% vs. 4%), stomatitis (3% vs. 1%), HTN (92% vs. 1%), venous thromboembolism (5% vs. 3%)

Abbreviations: AEs = adverse events; 5-FU = 5-fluorouracil; FOLFOX4 = oxaliplatin, 5-FU, and leucovorin; GI = gastrointestinal; HTN = hypertension; mCRC = metastatic colorectal cancer; ORR = overall response rate; OS = overall survival; PFS = progression-free survival.

FOLFIRI (Figure 5). At data cutoff, median follow-up was 22.28 months, and 863 patients had died. In the aflibercept arm, patients experienced significant improvement in median OS, PFS, and overall response rate (ORR) vs. placebo.[57] Prespecified subgroup analyses demonstrated that the overall results were irrespective of previous exposure to bevacizumab, age, sex, race, previous hypertension, number of metastatic sites, or location of primary tumor.[89] However, a greater treatment effect on OS with aflibercept was observed in patients with liver metastases only (hazard ratio, 0.649; 95.34% CI, 0.492-0.855; P = .0899).[89] Discontinuation because of AEs was 26.6% in the aflibercept arm and 12.1% in the placebo arm.[57] The most common reasons for discontinuation in the aflibercept arm were asthenia/fatigue, infections, diarrhea, hypertension, and venous thromboembolic events. Grade 3/4 AEs occurring with at least 2% greater incidence in patients treated with aflibercept vs. placebo were diarrhea, asthenia/fatigue, stomatitis/ulceration, infections, hypertension, gastrointestinal/abdominal pains, neutropenia/neutropenic complications, and proteinuria (Figure 6A and B).[57] In patients in the aflibercept arm with and without previous bevacizumab treatment, respectively, the incidence (grade 3/4) of hypertension (16.6% vs. 20.5%), hemorrhage (3.2% vs. 2.8%), and venous and arterial thromboembolic events (8.0% vs. 7.8% and 2.1% vs. 1.7%) were similar.[89]

Possible Role for Aflibercept in Therapy

As new therapies for second-line treatment of mCRC are introduced, it is important to develop an understanding of their potential role in treatment. Since bevacizumab is a standard treatment for second-line management of patients with mCRC, comparison of aflibercept with bevacizumab may provide some insight regarding the potential role for aflibercept. Although there is no prospective comparison of aflibercept with bevacizumab, examination of relevant clinical trials such as the phase III VELOUR trial described earlier for aflibercept and the E3200 and TML (ML18147) trials for bevacizumab may be helpful. The E3200 trial randomized patients previously treated with a fluoropyrimidine and irinotecan to the combination of oxaliplatin, 5-FU, and leucovorin (FOLFOX4) with (n = 286) or without (n = 291) bevacizumab or bevacizumab alone (n = 243).[90] The bevacizumab alone arm was closed early after an interim analysis showed inferior survival compared with the other 2 arms. In addition, 36% of patients experienced 1 or more grade ≥ 3 toxicity. The TML study investigated the efficacy and safety of bevacizumab with standard second-line chemotherapy in patients whose disease progressed after bevacizumab plus standard first-line chemotherapy.[91] The choice of oxaliplatin- or irinotecan-based second-line chemotherapy was dependent on first-line therapy.

In the VELOUR study, the addition of aflibercept to FOLFIRI resulted in significant improvement in OS and PFS and was unaffected by previous treatment with bevacizumab (Table 4). Selected side effects commonly observed with FOLFIRI (diarrhea, stomatitis, infection, neutropenia) were more common in patients treated with aflibercept, as were anti-VEGF AEs—ie, hypertension, mucosal bleeding, and proteinuria. Although infrequent, gastrointestinal perforation, hemorrhage, and arterial thromboembolism were also more common with aflibercept. Grade ≥ 3 AEs were more common with the addition of aflibercept compared with placebo (83.4% vs. 62.5%). In the E3200 study, bevacizumab monotherapy provided little benefit, but the addition of bevacizumab significantly improved OS and PFS with a significant increase in grade ≥ 3 hypertension, bleeding, vomiting, and neuropathy compared with FOLFOX4. In the TML study, the combination of bevacizumab with standard chemo-

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therapy significantly prolonged OS and PFS with no increase in AEs beyond disease progression.

Conclusion

Aflibercept is a multiple angiogenic factor trap that binds VEGF-A, VEGF-B, and PlGF. Clinical trials demonstrate effective antitumor activity with an acceptable safety and tolerability profile. In VELOUR and a phase II trial of patients with mCRC, no unexpected AEs were reported, and AEs observed were those typically associated with anti-VEGF agents, namely hypertension and proteinuria. The phase III VELOUR trial showed that patients with mCRC receiving aflibercept in combination with FOLFIRI experienced statistically significant improvements in OS, PFS, and ORR when compared with those receiving placebo after failure with an oxaliplatin-containing regimen. Furthermore, results of the phase III VELOUR trial demonstrated a 24% decrease in risk of disease progression, an improvement in response rate from 11% to 19.8% and in survival at 2 years from 19% to 28%. These benefits were consistent regardless of previous bevacizumab therapy. In conclusion, aflibercept represents a potential new option in combination with FOLFIRI in the treatment of mCRC.

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ARTICLES

Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis

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Tumour growth requires accompanying expansion of the host vasculature, with tumour progression often correlated with vascular density. Vascular endothelial growth factor (VEGF) is the best-characterized inducer of tumour angiogenesis. We report that VEGF dynamically regulates tumour endothelial expression of Delta-like ligand 4 (Dll4), which was previously shown to be absolutely required for normal embryonic vascular development. To define Dll4 function in tumour angiogenesis, we manipulated this pathway in murine tumour models using several approaches. Here we show that blockade resulted in markedly increased tumour vascularity, associated with enhanced angiogenic sprouting and branching. Paradoxically, this increased vascularity was non-productive—as shown by poor perfusion and increased hypoxia, and most importantly, by decreased tumour growth—even for tumours resistant to anti-VEGF therapy. Thus, VEGF-induced Dll4 acts as a negative regulator of tumour angiogenesis; its blockade results in a striking uncoupling of tumour growth from vessel density, presenting a novel therapeutic approach even for tumours resistant to anti-VEGF therapies.

Tumour growth depends on expansion of the host vasculature into the tumour, through the process of tumour angiogenesis[1]. The connection between tumour growth and angiogenesis prompted the development of several approaches to limit tumour angiogenesis and thus control tumour growth. The best validated of these approaches involves blockade of the VEGF pathway. Blockade of VEGF controls tumour growth in numerous preclinical models[2,3], and recent results show that potent blockers of VEGF can completely prevent tumour angiogenesis in some models, thereby severely inhibiting tumour growth[4]. The promise of VEGF-blocking approaches has recently been realized in the clinic, as a VEGF-blocking antibody has been shown to have important effects on tumour progression and overall survival in cancer patients[5,6]. However, despite the critical role for VEGF in tumour angiogenesis, it is also clear that in some cases tumour growth and angiogenesis can proceed even in the face of potent VEGF blockade[7–9]. Thus, additional angiogenesis-targeted therapies are necessary for tumours resistant to VEGF blockade.

Mouse genetic studies have demonstrated that, in addition to the VEGF pathway, other signalling pathways are also required for normal embryonic vascular development (for reviews, see refs 10–12), raising the possibility that these pathways may also be important during tumour angiogenesis. One signalling pathway implicated in vascular development by gene deletion studies is the Notch pathway[13]. On binding a transmembrane ligand from the Delta/Jagged families, Notch transmembrane receptors generally provide signals to guide cell fate decisions[14,15]. In particular, Delta-like ligand 4 (Dll4) is absolutely required for normal vascular development[16–18] and is strongly expressed in tumour vessels[17,19,20].

To determine whether the Dll4/Notch pathway has a role during tumour angiogenesis, we manipulated this pathway in experimental tumour models in mice using a variety of genetic and pharmacologic approaches. We report that the Dll4/Notch pathway is a critical negative regulator of tumour angiogenesis, acting to restrain excessive VEGF-induced vascular sprouting and angiogenesis. Increased Dll4/Notch activity resulted in decreased tumour vascular density, whereas blockade of activity resulted in markedly increased vessel density. Paradoxically, this increased vascularity seemed to be non-productive and resulted in decreased tumour growth, even for tumours that are resistant to anti-VEGF therapy. Our findings provide a striking example of an uncoupling of tumour growth from tumour vascular density, and support the model that the Dll4/Notch pathway normally acts as a negative regulator of angiogenic sprouting induced by VEGF or other pathways.

RESULTS

VEGF induces high expression of Dll4 in tumour vessels. To confirm and extend earlier reports that Dll4 expression was markedly and specifically induced in blood vessels during tumour angiogenesis[17,19,20], we used a combination of Dll4 detection approaches in two different tumour models. First, we exploited '*Dll4* reporter mice' in which a β-galactosidase reporter gene was driven by the *Dll4* promoter[17]. Lewis lung carcinomas implanted into these mice showed strong reporter-based staining of tumour vessels, apparently at higher levels than of vessels in surrounding normal tissue (Fig. 1a, b). At higher resolution, immunostaining for the β-galactosidase reporter protein and comparison with adjacent sections in which all vessels were immunostained with CD-31/PECAM antibodies revealed strong *Dll4* reporter expression in tumour blood vessels and relatively weak staining in adjacent subcutaneous and dermal blood vessels[17] (Supplementary Fig. 1). To confirm that the β-galactosidase/*Dll4* reporter (*Dll4–LacZ*) construct marked sites of Dll4 protein, we generated polyclonal antibodies to murine Dll4; these antibodies also immunostained tumour blood vessels selectively (Fig. 1c, d). These findings were further confirmed by *in situ* hybridization for *Dll4* messenger RNA, which revealed prominent expression in the vessels of C6 glioma tumours[19] (Supplementary Fig. 1). Thus, Dll4 is indeed specifically expressed in the tumour vasculature, particularly in the smaller vessels. Moreover, expression of Dll4 was dependent on continuous VEGF signalling, because blockade of VEGF with VEGF Trap, a recombinant soluble receptor that potently blocks VEGF-A and placental growth factor (PlGF)[4], caused a rapid and marked decrease in the expression of Dll4 by tumour vessels (Fig. 1e).

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Activating and blocking the Dll4/Notch pathway in tumours. To manipulate the Dll4/Notch pathway in tumours, we first exploited a retroviral approach to overexpress forms of Dll4, which we reasoned would serve as blockers or activators, in tumour cells. On the basis of previous studies that used soluble versions of Dll to inhibit Notch signalling[21], we generated retroviral vectors encoding a soluble dimerized version of Dll4 in which the extracellular region of Dll4 was fused to the human IgG1 Fc constant region (termed Dll4–Fc) as a presumed blocker, as well as full-length membrane-bound Dll4 that presumably would act as an activator; these constructs were transduced into rat C6 tumour cells to produce C6 Dll4–Fc and C6 Dll4 cells. Importantly, C6 tumour cells that overexpressed Dll4–Fc or Dll4 did not have different growth characteristics *in vitro* than control tumour cells (data not shown).

The expected changes on Notch signalling in the host (mouse) stroma of subcutaneously implanted C6 rat tumour cells were confirmed by quantitative messenger RNA analyses using probes specific for the mouse versions (to detect expression in the host stromal cells and not in the rat tumour cells) of three genes that are characteristic targets of Notch signalling (*HES1*, *HEY2* and *NRARP*)[22–27]. That is, in C6 Dll4–Fc tumours, host Notch signalling was consistently reduced as reflected by decreased levels of these target genes, whereas in C6 Dll4 tumours the Notch pathway was activated (Supplementary Fig. 3). Similarly, in co-culture studies in which the transduced rat tumour cells were mixed with human umbilical vein endothelial cells, expression levels of the *HES1*, *HEY2* and *NRARP* genes in the cultured endothelial cells (assayed using human specific probes so as to specifically detect the endothelial versions of these transcripts) were reduced by co-culture with C6 Dll4–Fc cells and induced by C6 Dll4 cells (Supplementary Fig. 4). Finally, treatment of cultured human umbilical vein endothelial cells with purified Dll4–Fc protein rapidly and consistently repressed Notch signalling, as reflected by decreased expression of these target genes (Supplementary Fig. 5).

Blockade of Dll4/Notch results in decreased tumour growth. To explore the effects of Dll4/Notch pathway manipulation in tumours, we next examined tumours derived from C6 Dll4–Fc and C6 Dll4 cells for their vascular morphologies and tumour growth rates. Strikingly, Dll4–Fc and full-length Dll4 seemed to promote reciprocal changes in the tumour vasculature: the vasculature in C6 Dll4–Fc tumours was much more highly branched and had more fine interconnections than that of control tumours (Fig. 2a–f). The leading front of the vessels was replete with sprouts and filopodia (Fig. 2e, h). In contrast, the vasculature in C6 Dll4 tumours was notably

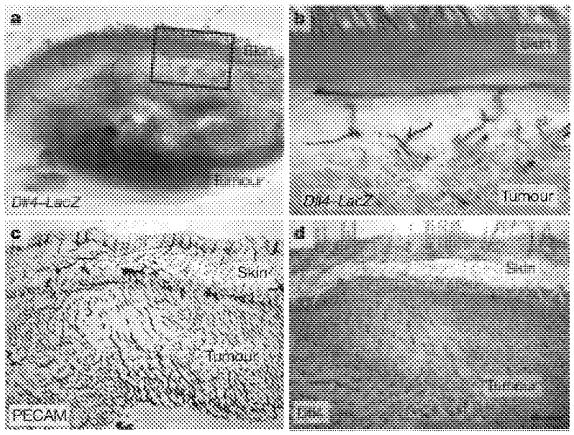


Figure 1 | Dll4 is expressed in tumour vessels, and its expression is dependent on VEGF signalling. **a**, **b**, Lewis lung tumours were grown subcutaneously in heterozygous *Dll4*-targeted mice in which the *Dll4* gene was replaced with the gene encoding β-galactosidase. **a**, *Dll4* expression, revealed by staining for LacZ (blue), was strong in tumour tissue, but weak in normal skin tissue. **b**, Higher magnification image showing Dll4 expression in tumour blood vessels. **c**, Section of tumour showing immunoreactivity for CD31/PECAM is equally strong in tumour vessels and adjacent skin vessels. **d**, In contrast, immunoreactivity for Dll4 is strong in tumour vessels and weaker in vessels of the adjacent skin. Scale bar: 400 μm (**c**, **d**). **e**, Expression of *Dll4* in tumour vessels is decreased by VEGF blockade. Rat C6 tumours grown subcutaneously were treated for the indicated times with VEGF Trap. Tumours were analysed for expression of murine *Dll4* by microarray analysis. Data show mean ± s.d. of *Dll4* expression compared with control C6 tumours from three tumours per time point ($n = 3$).

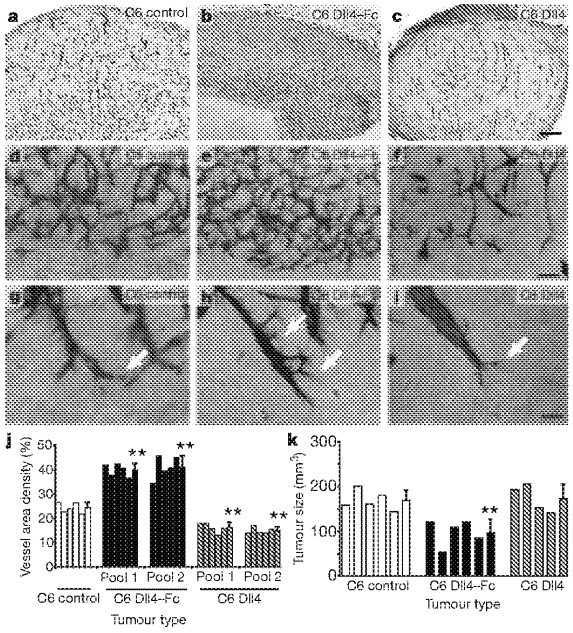


Figure 2 | Blockade of Dll4/Notch signalling results in smaller C6 tumours with increased vessel density. **a**–**i**, Micrographs showing tumours stained for CD31 (black) in control C6 tumours (**a**, **d**, **g**), in C6 tumours overexpressing Dll4–Fc (**b**, **e**, **h**), and in C6 tumours overexpressing full-length Dll4 (**c**, **f**, **i**). Micrographs show tumour sections at low, medium and high magnification. C6 Dll4–Fc tumours contain a dense network of vessel structures, with numerous cellular processes, particularly at the leading front (**b**, **e**). Reciprocally, C6 Dll4 tumours contain relatively sparse and unbranched vessels (**c**, **f**). The leading cells in the actively growing vascular front of Dll4–Fc tumours have more cellular processes than those in control tumours, and reciprocally, such cells in Dll4 tumours have fewer processes (arrows, **g**–**i**). Scale bars, 400 μm (**a**–**c**); 100 μm (**d**–**f**); 20 μm (**g**–**i**). **j**, Quantification of vessel area density by morphometry shows increased vessel density in C6 Dll4–Fc tumours and decreased vessel density in C6 Dll4 tumours. **k**, C6 Dll4–Fc tumours are smaller than C6 GFP control tumours, whereas C6 Dll4 tumours are the same size. Quantification was done on two independently isolated pools of C6 Dll4–Fc and C6 Dll4 cells (results from a single pool are shown). Data from individual tumours are shown, as well as mean ± s.d. (**j**, **k**) for each group ($n = 4$–5); $^{**}P < 0.01$.

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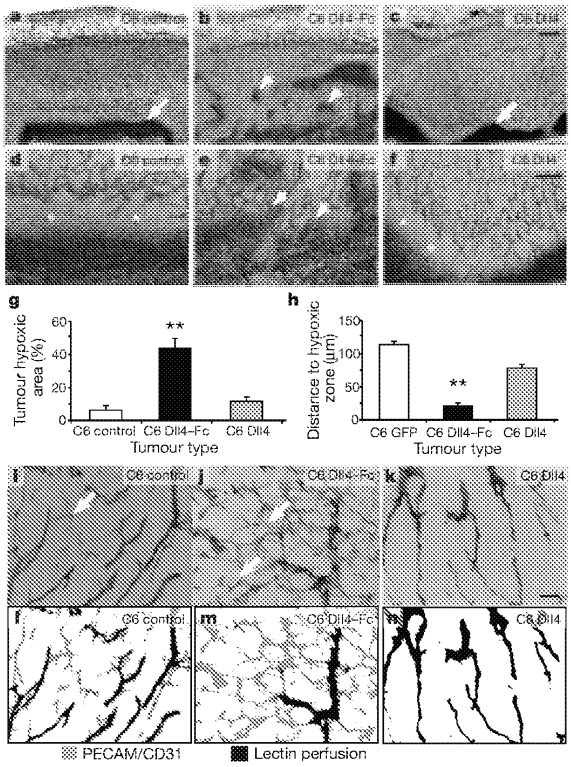


Figure 3 | Despite an increase in blood vessel density, tumours overexpressing Dll4–Fc have increased hypoxia and poor vascular perfusion. **a**–**f**, C6 tumours were stained for CD31/PECAM (brown) and for hypoxia (HypoxyProbe, black). Control C6 tumours of this size had no evident necrosis, and had very little hypoxia in the region of vascularized tumour, but did have a prominent rim of hypoxia at the base of the tumour (**a**, black staining, arrow) that was separated from the leading front of tumour vessels (asterisks in **d**). In comparison, C6 Dll4–Fc tumours contained increased areas of hypoxia in the region of vascularized tumour (arrowheads, **b**). In addition, hypoxia was found immediately adjacent to, or intermingled with, the leading front of tumour vessels (arrowheads, **e**). C6 Dll4 tumours had few areas of hypoxia in the region of vascularized tumour, and the hypoxic region (arrow, **c**) was typically separated from the leading front of vessels (asterisks, **f**), although more variability was seen than in control tumours. **g**, Quantification of hypoxic region within the vascularized tumour. C6 Dll4–Fc tumours contained significantly more hypoxia. **h**, Quantification of the distance between the leading front of vessels and the hypoxic zone, as marked with white asterisks in panels **d**, **e**, **f**. The distance to the hypoxic region was significantly less in C6 Dll4–Fc tumours than in control tumours. Values are mean ± s.d. (**g**, **h**) for each group ($n = 4$–5); $^{**}P < 0.01$. **i**–**n**, Comparison of vessel perfusion and overall CD31/PECAM immunoreactivity reveals decreased vessel function in C6 Dll4–Fc tumours. Tumour vessels were stained by *in vivo* intravascular injection of biotinylated lectin (stained in black) to mark perfused vessels, and by immersion in CD31/PECAM antibodies (stained in brown) to mark all vascular structures. **i**–**n**, Computer-generated colour images represent thresholds to show perfused vessels (black) and non-perfused vascular structures (green). Vascular structures in control C6 tumours (**i**, **l**) had a mixture of only brown staining, showing lack of perfusion (particularly in the fine processes, arrow) as well as a combination of black and brown staining showing perfused vessels. In C6 Dll4–Fc tumours (**j**, **m**), many vascular structures, particularly the numerous fine processes, were not perfused by intravascular tracer and thus stained only for CD31 (arrows). Reciprocally, in C6 Dll4 tumours (**k**, **n**), the unbranched vascular structures, which lack fine processes, were stained by both perfused lectin (black) and CD31 (brown), showing that vascular structures are functional. Scale bars, 100 μm (**a**–**c**); 40 μm (**d**–**f**); 20 μm (**i**–**n**).

straighter and relatively unbranched (Fig. 2c, f), and relatively devoid of sprouts and filopodia (Fig. 2f, i).

These obvious morphologic changes were reflected by quantitation of vascular area densities in these tumours, with the C6 Dll4–Fc tumours exhibiting increased vascular density as compared with control tumours, whereas the C6 Dll4 tumours exhibited slightly decreased vascular density (Fig. 2j). Paradoxically, the effects of Dll4–Fc and full-length Dll4 on vascular density were opposite to those that might be expected with respect to their effects on tumour growth. Despite increased vascular density, C6 Dll4–Fc tumours were consistently smaller than control tumours, whereas C6 Dll4 tumours were not substantially different in size from control tumours for small tumours (Fig. 2k) or when C6 Dll4 tumours were harvested at larger sizes (Supplementary Fig. 6). These results have two important implications: first, that the Dll4/Notch pathway normally serves as a negative regulator of sprouting and branching activity during tumour angiogenesis, so that blockade of this pathway results in increased tumour angiogenesis; and second, that the increased angiogenesis resulting from blockade of this pathway is in some sense 'non-productive', such that it does not support more robust tumour growth and instead seems to be associated with reduced tumour growth.

Blockade of Dll4/Notch increases tumour hypoxia. To account for the apparent paradox of increased tumour vessel density and decreased tumour growth in C6 Dll4–Fc tumours, we examined the possibility that the increased network of vessels might not be

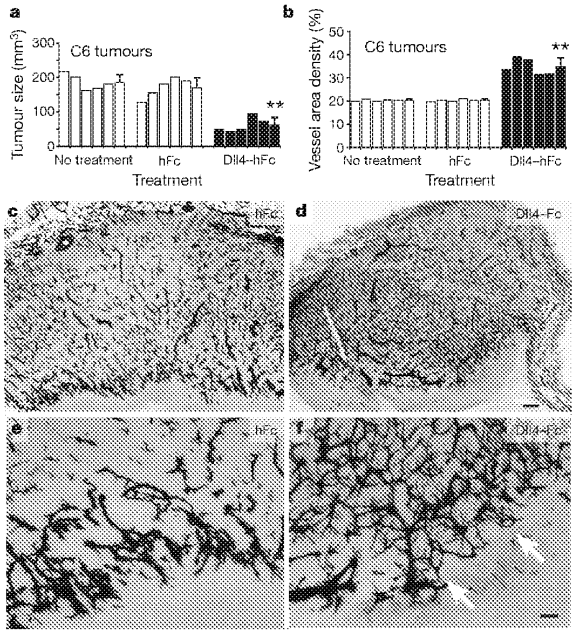


Figure 4 | Systemic delivery of Dll4–Fc using adenovirus results in smaller C6 tumours and increased vessel density, similar to effects of local tumour overexpression. **a**, C6 tumours were smaller in mice treated with systemically delivered Dll4–Fc, but not by control hFc. **b**, The vessel area density is increased in C6 tumours treated by systemic Dll4–Fc compared with untreated tumours or those treated with systemic hFc. The graphs in **a** and **b** show data from individual tumours, as well as mean ± s.d. for each group ($n = 5$). ** denotes significantly different than the control tumour group ($P < 0.01$). **c**–**f**, Micrographs showing tumour vessel morphology in control tumours (hFc; **c**, **e**) and in tumours treated systemically with Dll4–Fc (Dll4–Fc; **d**, **f**). The vessel density is increased in C6 Dll4–Fc tumours, particularly at the leading front (**f**, arrows). Tumour sections stained for CD31 (PECAM, black). Scale bars, 200 μm (**c**, **d**); 50 μm (**e**, **f**).

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optimally functional. In agreement with this possibility, histologic assessment showed more extensive tumour hypoxia in C6 Dll4–Fc tumours than in control tumours (Fig. 3a, b; hypoxic regions stained in black). Moreover, whereas the hypoxic region in control tumours was separated from the growing front of tumour vessels by an avascular zone that was itself not hypoxic (corresponding to the oxygen diffusion distance; white asterisks, Fig. 3d), areas of hypoxia were interspersed with the tumour vasculature in C6 Dll4–Fc tumours (arrowheads, Fig. 3b, e), indicating that this vasculature was not efficiently delivering oxygen to the surrounding tumour. Quantitative analysis showed that Dll4–Fc tumours contained seven-fold more total hypoxic area within the vascularized tumour (Fig. 3g), and that the hypoxic rim was less separated from the leading front of tumour vessels (Fig. 3h). Corresponding values in the C6 Dll4 tumours were not substantially different from controls (Fig. 3c), although there was more variability in the distance from the vascular front to the hypoxic rim (white asterisks, Fig. 3f).

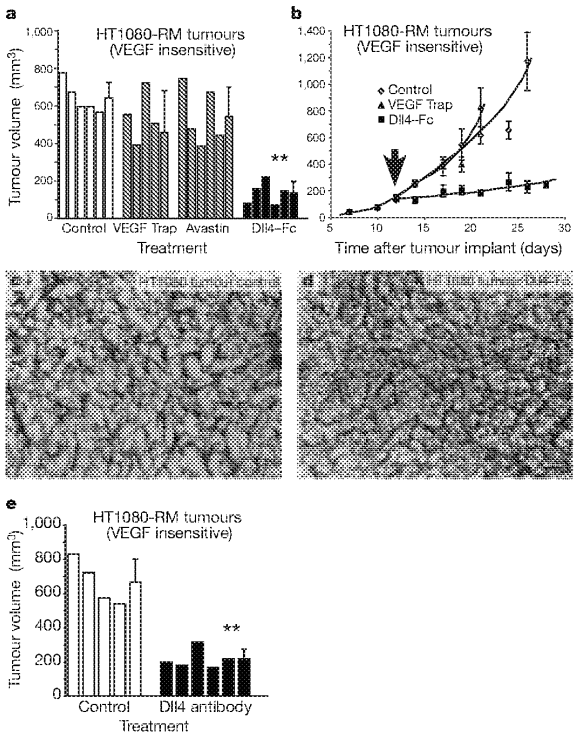


Figure 5 | Systemic delivery of Dll4–Fc or blocking Dll4 antibodies to mice bearing tumours that are resistant to blockade of VEGF results in decreased tumour growth and dramatic changes in tumour vessels. a, Size of HT1080-RM tumours treated with Dll4–Fc protein or VEGF Trap or bevacizumab (Avastin, all at 25 mg kg^{-1}). Tumours were treated with subcutaneous injections starting once tumours had reached approximately 100 mm^3. Dll4–Fc treated tumours were smaller than controls, whereas those treated with blockers of VEGF were almost the same size as controls. **b**, Tumour growth curves from a separate experiment in which HT1080-RM tumours were treated from 100 mm^3 (arrow) with VEGF Trap, Dll4–Fc, or hFc (25 mg kg^{-1}, three times per week). Error bars are s.d. Whereas tumours treated with VEGF Trap grew as rapidly as control tumours, those treated with Dll4–Fc were restrained for at least 2 weeks of treatment. **c**, **d**, Dll4–Fc caused an increase in tumour vessel density and dramatic changes in vessel morphology in HT1080-RM tumours. Tumour sections were stained for CD31. Scale bar, 50 μm (**c**, **d**). **e**, Antibodies to Dll4 strongly suppressed tumour growth, similar to the effect of Dll4–Fc. HT1080-RM tumours were treated from a size of 100 mm^3 with polyclonal antibodies to Dll4 (10 mg kg^{-1}, three times per week) or with rabbit IgG as a control. The bar graphs show data from individual tumours, as well as the mean ± s.d. for each group (n = 4–5).

The increase in tumour hypoxia in the C6 Dll4–Fc tumours suggested that the dense network of vessels was not fully perfused. We compared the distribution of vessel perfusion (marked by intravascular lectin as a tracer) with the immunohistochemical staining of endothelial cells (stained with CD31/PECAM-1 antibodies) as a marker of total vasculature. Most of the larger vessels of control tumours were perfused, although—as expected—these vessels were associated with some non-perfused sprouts and smaller vessels emanating from the larger vessels (Fig. 3i, brown reveals total vasculature, black reveals perfused vessels; Fig. 3l, computer-generated colour depiction, with green showing total vasculature and black showing perfused vessels). In contrast, many of the vascular processes in the C6 Dll4–Fc tumours were not perfused (Fig. 3j, m), suggesting that the increased vessel density seen in these tumours is not part of a functional vascular network. Reciprocally, the relatively straight and unbranched vessels seen in the C6 Dll4 tumours were almost completely perfused (Fig. 3k, n).

Together, the tumour hypoxia and perfusion analyses support the notion that the increased vascular network that results from inhibition of the Dll4/Notch pathway (by Dll4–Fc) is not optimally functional and is instead 'non-productive'.

Systemic Dll4–Fc decreases tumour growth. To confirm and extend the finding that locally produced Dll4–Fc promotes excessive angiogenesis that paradoxically blunts tumour growth, we used an adenoviral delivery approach to determine whether systemic Dll4–Fc could also produce this effect. Adenoviruses expressing Dll4–Fc, or human Fc (hFc) as control, were injected intravenously into mice at the time of implanting subcutaneous C6 tumours. The intravenously injected adenovirus infects hepatocytes, and in the case of Dll4–Fc or hFc, produced high serum levels of the encoded protein of 74 ± 20 μg ml^{-1} (range of 50–100 μg ml^{-1}, n = 5 mice). Circulating Dll4–Fc resulted in an approximately 70% reduction in the size of subcutaneous C6 tumours (Fig. 4a), whereas circulating control hFc had no effect. When examined by histology, circulating Dll4–Fc also caused an increase in the density of the tumour vessels (Fig. 4b). The overall increased vessel density produced by circulating Dll4–Fc was associated with a dense mesh of highly branched and sprouted vessels, particularly at the leading front (Fig. 4c–f), similar to that produced by Dll4–Fc overexpression in the tumour cells. Gene expression analysis confirmed that systemic Dll4–Fc suppressed the Notch pathway in the tumour vessel, as indicated by decreased expression of *HES1* and *NRARP* (data not shown). Thus, inhibition of Dll4/Notch signalling by either local or systemic Dll4–Fc results in smaller C6 tumours and excessive but apparently non-productive tumour angiogenesis. Importantly, the systemic treatment did not seem to have untoward effects on the host animals; in addition, preliminary analysis of normal tissues did not reveal obvious changes in tissue vascularity (Supplementary Information).

Dll4–Fc or Dll4-blocking antibody act in multiple tumour models. To further extend the above findings, we used systemic injection of purified recombinant Dll4–Fc protein as the treatment, and tried additional tumour models. The above studies were carried out using C6 gliomas, which are relatively sensitive to the effects of VEGF blockade[4], so we assessed the response to Dll4/Notch blockade in other tumours that are more resistant to VEGF blockade. In previous experiments using both bevacizumab (Avastin) and VEGF Trap, we had developed a model of HT1080 tumours (HT1080-(resistance model)RM), which is relatively resistant to both Avastin and VEGF Trap (G.T., I.N.-T. and J. Rudge, unpublished results). In contrast to Avastin and VEGF Trap, Dll4–Fc protein was quite effective in reducing the growth of HT1080-RM tumours (Fig. 5a). In a separate experiment (Fig. 5b), we assessed the growth curves of HT1080-RM tumours treated with Dll4–Fc, VEGF Trap or control protein (all at 25 mg kg^{-1}, three times per week; treatment began when tumours were approximately 100 mm^3 in size, arrow). Dll4–Fc

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treatment resulted in a prolonged suppression of tumour growth, whereas VEGF Trap had almost no impact on tumour growth in this resistant tumour model. Dll4–Fc treatment of HT1080-RM tumours also had a marked effect on tumour vessels. The already-dense vasculature of control HT1080 tumours (Fig. 5c) was further increased by treatment with Dll4–Fc (Fig. 5d), inducing an apparent increase in vascular sprouting and branching, and an apparent disorganization to the network.

To verify the specificity of blocking with Dll4–Fc, we also generated polyclonal antibodies to the extracellular portion of Dll4, which inhibited the binding of Dll4 to Notch1 in *in vitro* assays (Supplementary Fig. 7). Systemic treatment of mice bearing resistant HT1080-RM tumours with blocking Dll4 antibodies also reduced tumour growth (Fig. 5e) and caused marked changes in tumour vessels (data not shown).

As for HT1080 tumours, growth of mouse mammary tumours, which are also resistant to VEGF blockade (not shown), was strongly suppressed by systemic treatment with Dll4–Fc (Supplementary Fig. 8). Again, the vascularity of mouse mammary tumours was further increased by treatment with Dll4–Fc (Supplementary Fig. 8). Thus, as with C6 tumours, treatment of other tumours with systemic Dll4–Fc resulted in decreased tumour growth, accompanied by a denser and more highly branched tumour vasculature.

Discussion

To determine whether the Dll4/Notch pathway has a role during tumour angiogenesis, we manipulated this pathway in tumours using a variety of approaches. Our findings suggest that tumour-derived VEGF induces Dll4 expression in angiogenic endothelial cells as a critical negative regulator of vascular growth, acting to restrain excessive vascular sprouting and branching, and allowing angiogenesis to proceed at a productive rate (Supplementary Fig. 9). Thus, increasing Dll4/Notch activity resulted in decreased vascular density associated with less sprouting and branching of the vascular network. In contrast, Dll4/Notch blockade was associated with enhanced angiogenic sprouting and branching, resulting in a marked increase in tumour vessel density but a decrease in vessel function (Supplementary Fig. 9). Previously, angiogenesis-based treatment of tumours has focused on trying to block angiogenesis; however, our results using Dll4 blockade suggest an alternative approach based on promoting 'non-functionality' in the growing tumour vasculature. Although our studies suggest that VEGF blockade may be equally or more effective than Dll4 blockade in many tumour models, certain models that are resistant to VEGF blockade can still be sensitive to Dll4 blockers.

We, and others, have shown that Dll4 is specifically expressed in remodelling vessels and is the major Notch ligand in the vasculature. Thus, rather than a general blockade of the Notch pathway, specific blockade of Dll4 may lead to more specific disruption of tumour growth without significant impairment of Notch function in normal host tissues, and thus might be well tolerated in long-term treatments. It seems likely that biological therapeutic agents, which can be specific to a particular ligand or receptor in this complex pathway, may prove more potent and specific than more general pathway blockers, such as the γ-secretase inhibitors that not only block all Notch signalling but also other important γ-secretase-mediated signalling as well.

Our findings provide a striking example of an uncoupling of tumour growth from tumour vascular density. Although a large literature supports the notion that tumour growth rate may correlate with tumour vascular density, other studies argue that tumour angiogenesis must be regulated to be productive. For example, a recent study suggests that tumours may have higher vascular densities than is necessary to support their growth, and thus tumour angiogenesis may often exceed an optimally productive rate[28]. Consistent with the concept of excessive tumour vessel density, some recent studies suggest that pruning of the vasculature might actually improve tumour perfusion and oxygenation[29–31]. The present studies with blockers of Dll4/Notch seem to provide the other side of the argument; in particular, that Dll4 blockade may further compromise tumour vasculature function by causing excessive non-productive angiogenesis, which can in turn inhibit tumour growth. The overall message seems to be that even tumour vascular networks require a regulated balance of growth factors to generate a hierarchy of well-organized and well-functioning vessels. VEGF clearly has a key angiogenic role in a wide variety of tumours, but Dll4 blockade may present a new therapeutic opportunity in cancer, and one that might be beneficial for patients with tumours that are resistant to anti-VEGF therapies.

METHODS

Engineered retroviruses. Retroviruses engineered to express green fluorescent protein (GFP) (control), GFP plus Dll4–hFc or GFP plus Dll4 were used to transduce C6 rat glioma tumour cells. Tumour cell pools, sorted by flow cytometry, were implanted subcutaneously in severe combined immunodeficient (SCID) mice (8–10 weeks old). Tumours were harvested and processed for histology and/or gene expression analysis.

Engineered adenoviruses. Adenoviruses engineered to express hFc or Dll4–Fc were injected into the jugular vein of mice bearing subcutaneous C6 glioma, mouse mammary or HT1080 tumours. Adenoviruses provided systemic production of the engineered proteins.

Antibodies. Anti-Dll4 polyclonal antibodies were generated by immunizing rabbits against murine Dll4–hFc protein. Serum was depleted for antibodies with reactivity to human Fc and then used to stain tumour sections or treat tumour-bearing mice.

Reporter mice. Dll4 Lac/Z reporter mice[17] generated using Velocigene technology[32] were implanted with Lewis lung tumour cells. Tumours were stained with antibodies to CD31/Pecam-1 and/or β-galactosidase, or reacted with 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal), and counterstained with pyronin-Y.

***In vitro* assays.** *In vitro* assays to determine the effect of Dll4–Fc and full length Dll4 on Notch signalling used confluent human umbilical vein endothelial cells (VEC Technologies) treated with Dll4–Fc protein (10 μg ml^{-1}) for 2 to 8 h. RNA was extracted and analysed by Taqman, using probes and primers specific for human HES1, HEY2 and NRARP. In other experiments, human umbilical vein endothelial cells (50% confluent) were co-cultured with C6 glioma cells and assayed at 24 h.

Assaying hypoxia and vessel perfusion. To measure hypoxia and vessel perfusion, HypoxyProbe-1 (Chemicon; 60 mg kg^{-1}) was injected intraperitoneally one hour before sacrifice. Tumours were processed for histological analysis, and tumour sections were stained using anti-Hypoxyprobe antibody. To mark vessel perfusion, mice were injected through the jugular vein with biotinylated *Lycopersicon esculentum* lectin (100 μg, Vector Laboratories). Lectin circulated for 3 min, and then tumours were subsequently stained for lectin bound to the endothelial cell surface[33].

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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VEGF Trap complex formation measures production rates of VEGF, providing a biomarker for predicting efficacious angiogenic blockade

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Contributed by George D. Yancopoulos, September 21, 2007 (sent for review July 16, 2007)

VEGF is the best characterized mediator of tumor angiogenesis. Anti-VEGF agents have recently demonstrated impressive efficacy in human cancer trials, but the optimal dosing of such agents must still be determined empirically, because biomarkers to guide dosing have yet to be established. The widely accepted (but unverified) assumption that VEGF production is quite low in normal adults led to the notion that increased systemic VEGF levels might quantitatively reflect tumor mass and angiogenic activity. We describe an approach to determine host and tumor production of VEGF, using a high-affinity and long-lived VEGF antagonist now in clinical trials, the VEGF Trap. Unlike antibody complexes that are usually rapidly cleared, the VEGF Trap forms inert complexes with tissue- and tumor-derived VEGF that remain stably in the systemic circulation, where they are readily assayable, providing unprecedented capability to accurately measure VEGF production. We report that VEGF production is surprisingly high in non-tumor-bearing rodents and humans, challenging the notion that systemic VEGF levels can serve as a sensitive surrogate for tumor load; tumor VEGF contribution becomes significant only with very large tumor loads. These findings have the important corollary that anti-VEGF therapies must be sufficiently dosed to avoid diversion by host-derived VEGF. We further show that our assay can indicate when VEGF is optimally blocked; such biomarkers to guide dosing do not exist for other anti-VEGF agents. Based on this assay, VEGF Trap doses currently being assessed in clinical trials are in the efficacious range.

aflibercept | angiogenesis | tumor | endothelial cell

VEGF is critical in many settings of physiological and pathological angiogenesis (1). In particular, high VEGF expression is characteristic of many types of cancers (1), suggesting that it might be an attractive target for therapeutic intervention aimed at preventing tumors from recruiting the blood supply that they need to survive (2). The first attempts at validating this particular approach were taken by Ferrara and colleagues (3), who demonstrated that a murine anti-human VEGF antibody suppressed the growth of human tumor cell lines implanted in nude mice. This led to the generation of a humanized monoclonal antibody, bevacizumab (Avastin; Genentech, South San Francisco, CA), which yielded impressive results in a controlled clinical trial in patients with metastatic renal cell cancer (4, 5). At doses of 3 and 10 mg/kg, bevacizumab treatment resulted in a significant prolongation in time to tumor progression compared with placebo, although the increased efficacy of the higher dose in this study suggested that the maximally efficacious dose may not yet have been attained (4, 5). Bevacizumab was subsequently granted FDA approval based on the demonstration that it significantly improved the progression-free and overall survival in patients with metastatic colorectal cancer when given in combination with irinotecan 5-FU/LV chemotherapy (6). Several other drugs designed to block VEGF signaling have since been developed and recently approved [BAY 43–9006 (sorafenib) and SU11248 (sunitinib)] or are proceeding through clinical trials [PTK787 (vatalanib), ZD6474 (zactima), ZD6126, SU5416 (semaxanib), and AG-013736] (7–9).

As new anti-VEGF agents proceed through the clinic, it would be very useful to have biomarkers that could either identify patients whose tumors depend most on VEGF or that could guide dosing by indicating when optimal VEGF blockade has been achieved. Unfortunately, accepted biomarkers do not currently exist for VEGF blockade and are few and far between for other targeted agents, such as epidermal growth factor receptor for colon cancer, Kit for gastrointestinal stromal tumor, and HER2/NEU for breast cancer (10). VEGF itself has been suggested as a candidate biomarker for guiding the application of anti-VEGF therapies. It is widely assumed that VEGF production is quite low in healthy adults in the absence of active angiogenesis. Were that the case, blood levels of VEGF in cancer patients might provide a useful index of tumor VEGF production (11, 12). However, because VEGF is rapidly cleared from the systemic circulation (having a half-life of only minutes), the sensitivity of assays measuring VEGF in the peripheral blood leads to a wide variability for blood levels of VEGF in published reports. Furthermore, VEGF is present at substantial levels within platelets and released upon their lysis such that preparation of peripheral blood samples that avoid contamination from platelet-derived VEGF becomes difficult. These limitations are reflected in the disparate values reported for circulating VEGF levels in cancer patients, which range from 0.04 to 1 ng/ml, calling into question the utility of plasma VEGF levels as a useful biomarker for guiding anti-angiogenic therapy (11, 13–19).

VEGF Trap is a fully human soluble decoy receptor protein that consists of a fusion of the second Ig domain of human VEGF receptor (VEGFR) 1 and the third Ig domain of human VEGFR2 with the constant region (Fc) of human Ig IgG1 (20).

Author contributions: J.S.R., J.H., and G.D.Y. designed research; J.S.R., S.J.W., G.T., and N.S. analyzed data; J.H., D.H., M.R., S.J., R.L., N.P., E.A.P., A.T., and G.T. performed research; N.P. contributed new reagents/analytic tools; and J.S.R. and G.D.Y. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

Abbreviations: AMD, age-related macular degeneration; MALLS, multiangled laser light scattering; SEC, size exclusion chromatography; VEGFR, VEGF receptor.

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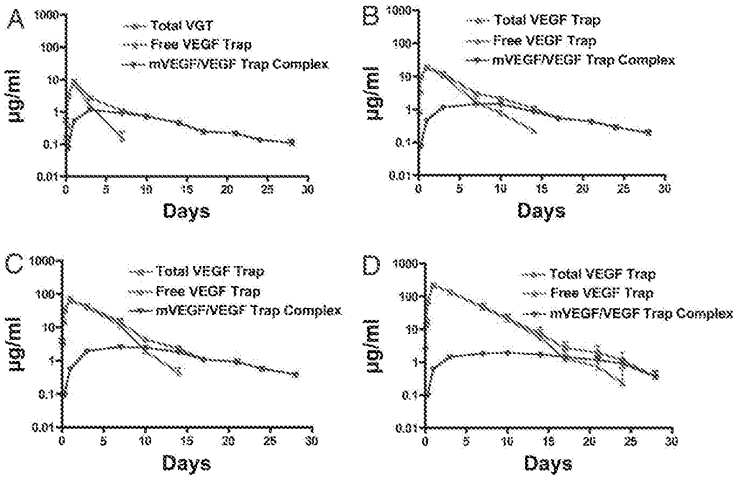


Fig. 1. s.c. injection of VEGF Trap into SCID mice at different doses reveals different levels of circulating free VEGF Trap but similar levels of circulating mouse VEGF–VEGF Trap complex. At all doses ranging from 1 mg/kg (*A*) to 25 mg/kg (*D*), a steady-state level of VEGF–VEGF Trap complex is achieved, which plateaus at ≈1 μg/ml. Dose-dependent levels of free VEGF Trap are observed as follows: 1 mg/kg to 10 μg/ml C_{max} falling below complex levels at 4 days (*A*); 2.5 mg/kg to 20 μg/ml C_{max} falling below complex levels at 7 days (*B*); 10 mg/kg to 80 μg/ml C_{max} falling below complex levels at 9 days (*C*); and 25 mg/kg to 200 μg/ml C_{max} falling below complex levels at 17 days (*D*). The half-life of VEGF Trap is ≈2 days at doses >2.5 mg/kg. (n = 6 for each dose.)

The VEGF Trap was engineered to have optimized pharmacokinetic properties and a very high affinity for all isoforms of VEGF-A (<1 pM), as well as placental growth factor, a closely related angiogenic factor (20). VEGF Trap has shown robust antitumor effects in numerous mouse models of cancer and is now in clinical trials (21, ‡, §, ¶, ‖). Here, we show that—unlike VEGF antibodies that tend to form multimeric immune complexes that are rapidly cleared from the circulation and can form immune complex deposits in tissues—the VEGF Trap forms a stable and inert 1:1 complex with VEGF. This VEGF–VEGF Trap complex has a long plasma half-life and can readily be measured in the systemic circulation, thus affording a reliable way to measure the rates of VEGF production in both tumor-bearing and non-tumor-bearing adult animals and humans. This unique ability to capture and thus precisely measure total VEGF levels, regardless of whether the VEGF comes from tumor or normal host tissues, allows for the unprecedented opportunity to accurately determine tumor and host VEGF production rates. Surprisingly, we find that total body VEGF production rates are quite high in normal adult rodents and humans, with the fractional contribution made by tumors being comparatively small. This finding has the important implication that therapies directed toward neutralizing VEGF produced by tumors must be provided in sufficient amounts so as to avoid being largely consumed by the significant levels of VEGF produced by the rest of the body. Toward this end, measurement of VEGF Trap complex allows the identification of VEGF Trap doses required to completely capture and block tumor-derived VEGF, providing a useful guide for optimizing angiogenic blockade; such assays do not exist for other anti-VEGF agents. Based on this assay, we report that VEGF Trap doses currently being assessed in clinical trials appear to be in the efficacious range.

Results

VEGF Trap Forms an Inert Complex with VEGF That Remains Stably in the Circulation. Initial studies to determine the clearance rate of VEGF Trap revealed that it could form stable detectable complexes with endogenous VEGF in normal adult mice. After single injections of increasing amounts of VEGF Trap, we measured total VEGF Trap, uncomplexed/unbound or "free" VEGF Trap, and VEGF Trap–mouse VEGF "complex" at various times after injection (Fig. 1 *A–D* represent increasing amounts of injected VEGF Trap). Because no exogenous VEGF was provided, complexes represent the association of VEGF Trap with endogenous murine VEGF. As expected, total VEGF Trap levels increased proportional to dose (determined by combining free VEGF Trap levels with complex levels) (Fig. 1, see green curves). Somewhat unexpectedly, substantial levels of VEGF Trap complexed with mouse VEGF accumulated rapidly (Fig. 1, see blue curves). At all doses of VEGF Trap tested, maximal levels of complex (≈1–2 μg/ml) were attained within 24–48 h of injection and sustained at this level for at least several days. Consistent with conversion of free VEGF Trap into complexed VEGF Trap, most of the injected VEGF Trap is initially found in the free, unbound form, but after reaching peak levels (≈24 h after injection) free VEGF Trap in the circulation declines progressively (Fig. 1, note that red curves, corresponding to free VEGF Trap, initially overlap at early time points with green curves, representing total VEGF Trap, but then drop, as is most obvious at the lowest dose). Levels of free VEGF Trap decline because of a "consumption" (binding VEGF, thus being converted to complex) and clearance, which occurs at an identical rate for free and bound Trap. Thus, as long as free VEGF Trap remains in excess of bound, maximal steady-state levels of complex are maintained in the circulation. VEGF Trap is also able to bind placental growth factor with high affinity and is capable of forming stable circulating placental growth factor–VEGF Trap complexes *in vivo* with the same profile as VEGF–

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VEGF Trap complex, albeit at ≈10-fold lower levels (data not shown).

In separate experiments, the bioavailability of VEGF Trap and the efficiency of VEGF capture were determined by injecting s.c. [supporting information (SI) Fig. 7*A*] or i.v. (SI Fig. 7*B*) preformed complexes of the Trap and its VEGF target, or both agents separately. The results show that the bioavailability of s.c. (SQ) injected complex was essentially identical to that of i.v. injected complex, indicating that negligible complex was depositing within tissues. Moreover, whether the VEGF Trap was injected as a preformed complex with VEGF (single bolus) or the Trap and its target were injected separately, similar levels of complex were rapidly noted in the circulation, indicating that the Trap efficiently captures its target and brings it into the systemic circulation. In addition, VEGF Trap is also capable of sequestering VEGF already bound in target tissues as shown by injecting VEGF before VEGF Trap (SI Fig. 7). Thus, VEGF Trap efficiently captures and forms inert complexes with VEGF that enter and remain stably in the circulation, readily accessible for measurement.

Although VEGF Trap Forms a 1:1 Complex with VEGF, VEGF Antibodies Form Heterogeneous, Multimeric Immune Complexes with VEGF. The above findings suggested that VEGF Trap might behave very differently than VEGF antibodies, because antibodies commonly form multimeric immune complexes that rapidly deposit in tissues and thus are rapidly cleared from the circulation. Because immune complexes rapidly disappear, the amount of captured ligand cannot be determined from levels of bound or unbound antibodies remaining in the circulation. To demonstrate directly that the VEGF Trap behaves in a fundamentally different way than antibodies, we compared VEGF Trap complex formation and clearance with that of a well characterized VEGF antibody, bevacizumab (Avastin). As predicted, size exclusion chromatography (SEC) of a preformed VEGF Trap–$VEGF_{165}$ complex revealed a single major homogenous peak, with an approximate molecular mass (as judged by comparison to molecular mass standards, data not shown) of ≈150 kDa corresponding to that expected of a 1:1 complex between VEGF Trap (≈110 kDa) and $VEGF_{165}$ (≈40 kDa) (Fig. 2*A*, solid red line); a minor peak of free excess $VEGF_{165}$ was also seen, as was a small shoulder of higher molecular mass. The molecular masses of the peaks were confirmed by using coupled multiangled laser light scattering (MALLS) (dashed red lines in Fig. 2*A*). In contrast, SEC of preformed bevacizumab–$VEGF_{165}$ complexes revealed a heterogeneous mixture corresponding to very high molecular masses (Fig. 2*A*, solid blue line) in addition to the small peak of free excess $VEGF_{165}$. The purity of free VEGF Trap, bevacizumab, and VEGF was >97%, as determined by SEC (data not shown). Coupled MALLS analysis revealed molecular masses of the heterogeneous mixture ranging from 370 kDa (corresponding to a multimer consisting of two bevacizumab molecules, each with a molecular mass of ≈145 kDa, and two VEGF165 molecules, each with a molecular mass of ≈40kDa) to >2,000 kDa (corresponding to much larger multimers) (Fig. 2*A*, dashed blue line). Consistent with the apparent tendency of bevacizumab to form multimeric immune complexes with VEGF, preformed bevacizumab–$VEGF_{165}$ complexes rapidly disappeared from the circulation when injection intravenously, as would be expected for multimeric immune complexes (SI Fig. 8; note that the levels of Bevacizumab when complexed with VEGF rapidly drop compared with the levels of free Bevacizumab that remain much higher), and in contrast to what was described above with VEGF Trap complexes that remain stably in the circulation. Because immune complexes can often be cleared by depositing in the renal glomeruli, we further explored apparent differences in the clearance of bevacizumab–VEGF and VEGF Trap–VEGF complexes by performing immunostaining in the kidney. After i.v. administration, renal glomeruli stained strongly for bevacizumab–VEGF complexes (Fig. 2 *D* and *E*) but not for VEGF Trap–VEGF complexes (Fig. 2 *B* and *C*). Current evidence indicates that, as a class, pharmacological agents that block VEGF signaling may produce mechanism-based effects on kidney function. Deposition of immune complexes as noted for bevacizumab/VEGF in the renal glomeruli could further accentuate renal toxicity in a nonspecific and non-class-dependent manner.

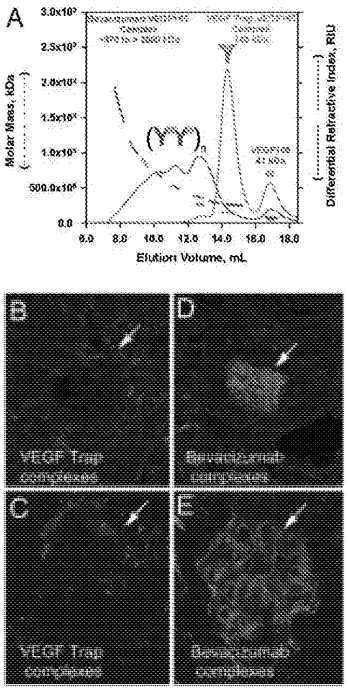


Fig. 2. The molar masses of VEGF Trap–VEGF and bevacizumab–VEGF complexes were determined by MALLS coupled to SEC. (*A*) Using a 1:2 molar ratio of VEGF Trap to $VEGF_{165}$, discrete peaks were observed at ≈17 ml for VEGF (41 kDa) and ≈14.5 ml for VEGF Trap–VEGF complex (148 kDa) with SEC (red line) and MALLS (dashed red line). In contrast, a 1:2 molar ratio of bevacizumab to $VEGF_{165}$ revealed a heterogeneous multimeric complex that ranged in molar mass from ≈370 kDa to >2,000 kDa (SEC, solid blue line; MALLS, dashed blue line). (*B*–*E*) One milligram of a preformed complex of VEGF Trap and $VEGF_{165}$ (*B* and *C*) or bevacizumab and $VEGF_{165}$ (*D* and *E*) were injected into the left ventricle of 2- to 3-month-old C57bl6 mice. After 10 min, mice were killed, and their kidneys were processed for immunocytochemistry, using an anti human Fc reporter antibody to the human Fc moiety present on both VEGF Trap and bevacizumab. Significant staining was observed in the glomeruli of bevacizumab/VEGF treated mice but not in the glomeruli of VEGF Trap/VEGF treated mice (white arrows).

VEGF Trap Complex Formation Reveals Unexpectedly High Production of Endogenous VEGF in Normal Adult Mice. As shown above, VEGF antibodies form immune complexes that rapidly deposit in tissues and thus do not allow for easy ascertainment of the amount of complex formed. In contrast, VEGF Trap forms inert complexes with VEGF that remain stably in the circulation and are thus readily accessible for measurement. In fact, the above findings demonstrate that, if VEGF Trap is present at sufficient levels so as to be in excess of Trap bound in complexes, the steady-state levels of VEGF Trap complex in the circulation reflect the total amount of VEGF produced. Daily production

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rates of VEGF can be calculated by assuming that steady-state levels of VEGF Trap–VEGF complex reflect a balance between production of VEGF leading to formation of complex, and clearance of the resulting complex. Based on experimentally determined values for the steady-state levels of complex and its clearance (see *Materials and Methods*), we estimate that mice produce ≈0.065 μg of VEGF per day per ml of the volume of distribution, or ≈0.006 μg per gram of tissue per day. Because VEGF is active at picomolar levels, this at first seems to be a surprisingly high level of production for a normal adult animal (see below for comparison to tumor production rates). However, it should be noted that in the absence of VEGF Trap, any VEGF that enters the systemic circulation is rapidly cleared. For this reason, among others noted above, it has not proven possible to consistently and reliably measure systemic VEGF levels, preventing accurate estimation of VEGF production rates in normal adult animals.

Tumor-Derived VEGF Represents a Minority of Total Body VEGF Under Conditions of Minimal Tumor Burden. Next, we compared the total body production rate of VEGF, as determined above, with tumor production rates of VEGF. Toward this end, we implanted mice with tumors, allowed these tumors to grow to 0.5–3% of total body weight (average mouse weight, ≈25 g) and measured levels of VEGF Trap complex in these mice to compare them to complex levels found in healthy, non-tumor-bearing mice. Surprisingly, in mice bearing four different types of rodent tumors, the total levels of complex were not markedly different from those seen in non-tumor-bearing mice (1–2 μg/ml; see Fig. 3*A* and compare with Fig. 1). This finding implies that tumor-derived VEGF represented only a small proportion of total body VEGF or circulating bioavailable VEGF in these mice.

To further validate this unanticipated finding, we analyzed VEGF Trap complex levels in mice bearing human tumors, where it is possible to distinguish complexes formed with endogenous mouse VEGF with those formed with human VEGF derived from the implanted tumors by analyzing human VEGF–VEGF Trap complex levels in mouse serum. The levels of mouse-derived complexes (Fig. 3*B*) in these animals were equivalent to those of non-tumor-bearing mice (Fig. 2, above) and mice bearing rodent-derived tumors (Fig. 3*A*). In contrast, the levels of VEGF Trap complexed with tumor-derived human VEGF were an order of magnitude lower (0.08–0.2 μg/ml) (Fig. 3*B*). This result was seen in mice bearing tumors of three different human cell lines (SK-NEP, A673, and HT1080). Together, these studies demonstrate that normal total body production of VEGF eclipses the production from tumors that may weight as much as 3% of body weight (mouse weight ranges from 23 to 29 g). Thus, it is unlikely that total levels of free VEGF in the systemic circulation would provide a sensitive index of tumor burden, even if accurate measurement of unbound VEGF in blood samples were readily achievable. Moreover, the above findings suggest that therapeutic compounds designed to bind and inactivate tumor-derived VEGF would have to be provided at sufficient levels to avoid being diverted by significant levels of VEGF normally produced by the rest of the body.

VEGF Trap Complex Levels Provide Guidance on When Efficacious VEGF Blockade Is Achieved. Based on the results above, it is evident that drugs that bind and neutralize VEGF must engage significant levels of VEGF derived from normal tissues, in addition to that originating from tumors. Therefore, we reasoned that measurements of VEGF Trap complex might provide a useful guide to when the dose of VEGF Trap sufficient to substantially neutralize both host and tumor-derived VEGF had been achieved. Indeed, for three different tumors [B16F1 mouse melanoma (Fig. 4*A*); A673 human rhabdomyosarcoma (Fig. 4*B*); and MMT mouse mammary carcinoma (Fig. 4*C*)], increasing the VEGF

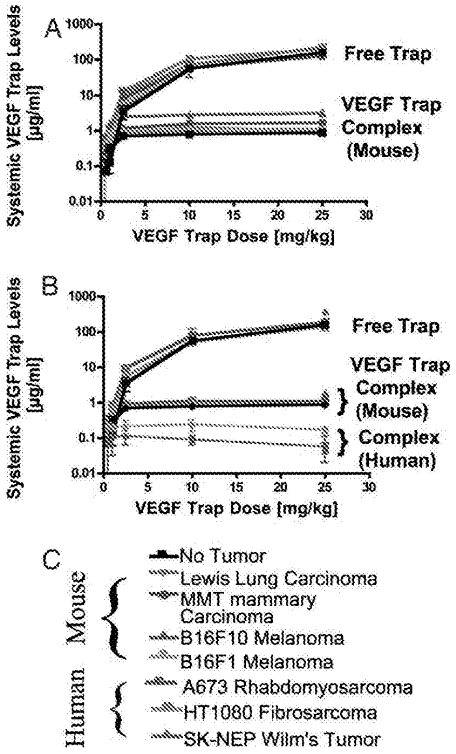


Fig. 3. In mice bearing tumors <3% body weight, the tumor pool of VEGF production is modest compared with endogenous mouse tissue VEGF production. (*A* and *B*) Mouse (*A*) or human (*B*) tumors were allowed to grow to ≈100 mm^3, and then VEGF Trap was administered twice per week for 1–2 weeks at 0.5, 1, 2.5, 10, and 25 mg/kg. At the termination of the experiment, free VEGF Trap, mouse, and human complex levels were measured in serum. In all cases, regardless of terminal tumor volume, levels of circulating mouse complex were ≈1 μg/ml, whereas human complex levels in the mice bearing human tumors were ≈0.1 μg/ml. Free Trap levels increased incrementally, with the dose levels rising above complex levels at the 2.5 mg/kg dose and reaching ≈100 μg/ml at the 25 mg/kg dose. ($n = 6$ for each dose). (*C*) Legend of mouse and human tumor types used.

Trap dose resulted in progressive, marked improvements in anti-tumor efficacy until a dose at which free VEGF Trap substantially exceeded maximal steady-state levels of complex was reached (Fig. 4). For all three tumor types, this was achieved at a dose of 2.5 mg/kg VEGF Trap given twice weekly: at this dose, free VEGF Trap (blue curve) is severalfold the level of complex (green curve), and past this point further dose escalation yields only modest incremental increases in complex levels (green curve) and in anti-tumor efficacy (red curve). In other tumor types, such as U87 glioblastoma, higher levels of VEGF Trap are required to achieve maximal efficacy (22).

Human VEGF/VEGF Trap Complex Levels Are Directly Related to Tumor Size. The finding that conventionally sized s.c. tumors in mice produced <10% the amount of total body VEGF prompted us to determine whether there is a consistent relationship between tumor size and VEGF production levels. Human tumors (A673 rhabdomyosarcoma) were implanted into mice and allowed to grow to various sizes before injecting VEGF Trap. In this case, we could define a clear linear relationship between tumor size (Fig. 5*A*) and complex levels (Fig. 5*B*, note that the assay reflects

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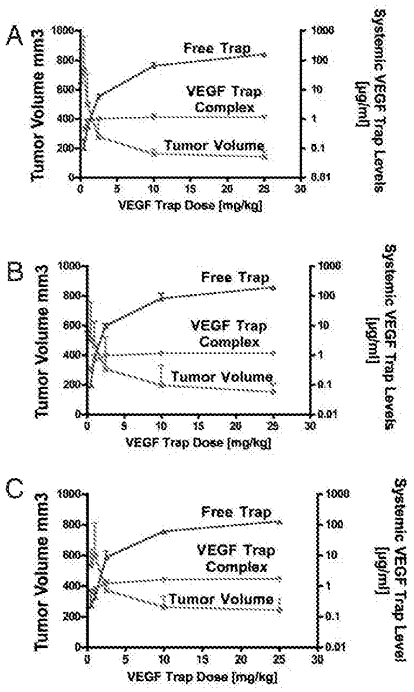


Fig. 4. VEGF Trap Complex provides guidance on when optimal VEGF blockade is achieved for antitumor purposes. In mice bearing B16F1 mouse melanoma tumors (*A*), A673 human rhabdomyosarcoma (*B*), and MMT mouse mammary carcinoma tumors (*C*) grown to ≈100 mm^3 before treatment, increasing the dose of VEGF Trap from 0.5 mg/kg twice per week to 25 mg/kg twice per week results in a steady-state of mouse complex at ≈1 μg/ml at 1–2.5 mg/kg and free circulating VEGF Trap levels of ≈10 μg/ml at the 2.5 mg/kg dose, rising to ≈100 μg/ml at the 25 mg/kg dose. Tumors remain quite large at the 0.5 and 1 mg/kg doses but begin to show a significant lack of growth at the 2.5 mg/kg dose, where free Trap levels rise above steady-state complex levels (n = 6 for each dose). Tumors were treated with VEGF Trap from 6–13 (B16F1), 4–13 (MMT), and 12–18 (A673) days after implantation.

levels of complexes containing only human VEGF to specifically detect only tumor-derived complex). The amount of complex per unit weight of tumor was similar across different-sized tumors (Fig. 5*C*), indicating that tumors maintained their rates of VEGF production as they grew. Linear regression analysis confirmed that there was a very strong correlation between A673 tumor volume and circulating human VEGF complex (Fig. 5*D*).

At these larger tumor sizes, the amount of complex (ranging from ≈0.8 to 5 μg/ml) contributed by the tumor matched or even exceeded that contributed by the rest of the body, confirming that tumors do indeed make substantially more VEGF per cell than does the average cell in the normal adult host. For example, in the largest tumors (weighing ≈10% of the total mass of the mouse, Fig. 5*A*), the tumor-derived human VEGF–VEGF Trap complex levels (≈5 μg/ml, Fig. 5*B*) were ≈3-fold above the levels of murine VEGF–VEGF Trap complex, indicating that the tumors made ≈30 times the amount of VEGF per unit of weight compared with normal, adult tissues.

VEGF Trap Complex Formation in Human Subjects With and Without Cancer. Very large tumors that substantially contribute to VEGF Trap complex formation in mice are generally not seen in the human patient. This in turn suggests that it is unlikely that most tumors in human patients become large enough to make a readily detectable contribution to total body VEGF production. To determine whether or not this was indeed the case, we studied VEGF Trap complex formation in non-cancer patients [patients suffering from age-related macular degeneration (AMD)] and then compared these results with complex formation in cancer patients. In the AMD patients, the lowest dose of VEGF Trap tested (0.3 mg/kg, i.v.) was insufficient to neutralize all VEGF, as evidenced by the levels of free Trap quickly falling below those of bound VEGF Trap, and bound VEGF Trap did not approach the maximal steady-state levels seen with higher doses (Fig. 6 *A* and *B*). However, doses of 1.0 and 3.0 mg/kg (i.v.) maintained substantial free Trap levels throughout the dosing period (Fig. 6*A*), and maximal complex levels were attained, as evidenced by equivalent levels of complex being generated at the two higher doses (≈1–2 μg/ml, see Fig. 6*B*). In cancer patients with advanced solid tumors or non-Hodgkin's lymphoma, remarkably similar results were obtained. That is, similar doses of VEGF Trap were required to saturate VEGF binding and complex formation (Fig. 6 *C–E*). In addition, the maximal steady-state levels of VEGF–VEGF Trap complex were similar to those seen in non-cancer patients (Fig. 6 *B*, *D*, and E). These findings indicate that, consistent with our findings in mice, endogenous VEGF production in adult human subjects is quite high, whether or not the individuals harbor tumors (Fig. 6*E*).

Using the same approach as was used for the mouse (see *Materials and Methods*), human production rates of VEGF in humans were found to be ≈0.0025 μg per gram of tissue per day, which is remarkably similar to that calculated for mice (see above). If our findings in animal models continue to be predictive, these VEGF Trap levels achieved in ongoing clinical studies should be in the efficacious range.

Discussion

At present, there are a number of anti-angiogenic agents targeting the VEGF pathway that are proceeding through clinical trials or already approved for the treatment of cancer (9). One major challenge is the lack of objective measures to guide dosing to determine when sufficient blockade has been achieved or to inform pharmacological response to these drugs. VEGF itself has been suggested as a potential biomarker for the above purposes, based on the assumption that VEGF in the peripheral circulation was primarily derived from the tumor and therefore accurately reflected tumor burden (19). However, to date it has proven difficult to accurately measure systemic levels of VEGF, correlate these levels with tumor burden, or use them as a guide to dosing (11, 12). Here, we describe the use of the VEGF Trap, a potent VEGF antagonist that forms a stable, inert complex with VEGF, as an index that allows for the accurate assessment of VEGF production rates. In addition, this unique property of the VEGF Trap allows accurate assessment of the amounts of VEGF made by a resident tumor compared with the rest of the body. Furthermore, in animals, this approach has been shown to provide a useful guide to selecting dosing regimens that substantially block available VEGF. This has not been possible with anti-VEGF antibodies, as VEGF–antibody complexes are rapidly cleared.

We find unexpectedly high levels of VEGF production in the normal adult setting, where it has long been assumed that, in the absence of ongoing angiogenesis, VEGF production rates would be quite low (11, 12). However, the unexpectedly high rates of VEGF production in non-tumor-bearing adult mice and humans is consistent with the recent realization that VEGF likely plays an ongoing role in the "quiescent" vasculature of normal adults (23). For example, treating normal adult mice and monkeys with VEGF antagonists can increase hematocrit (a measure of the proportion of the blood volume occupied by red blood cells) (24). Similarly, VEGF antagonists can also increase blood pressure (25), indicating that VEGF is involved in regulating

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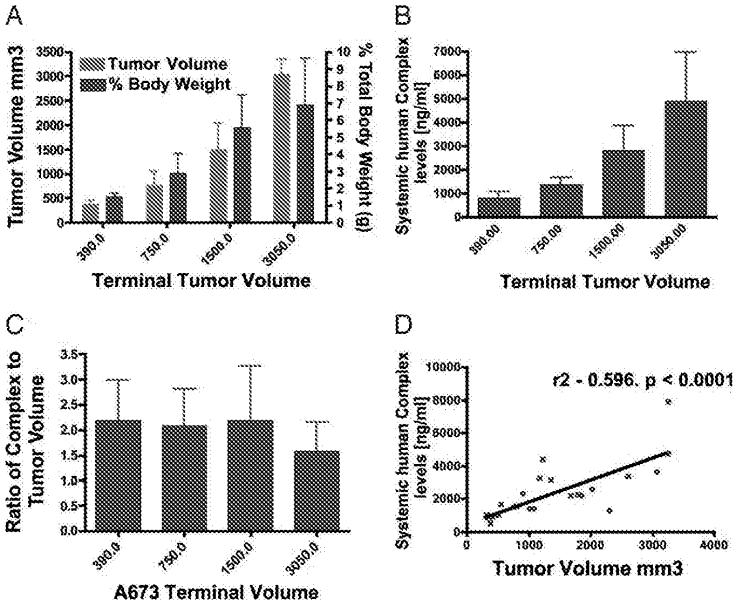


Fig. 5. Human VEGF–VEGF Trap complex levels are directly related to tumor size. Human A673 rhabdomyosarcoma tumors were grown in mice to ≈100, ≈300, ≈500, and ≈750 mm^3, at which point they were treated with a single bolus of 25 mg/kg. VEGF Trap, tumor volume, and human complex levels were measured after 2 weeks ($n = 6$). (*A*) Increasing tumor volume equates with an increase in tumor burden. (*B*) Increasing human tumor burden is reflected in an increase in circulating human VEGF–VEGF Trap complex. (*C*) The ratio of human VEGF–VEGF Trap complex to tumor volume remains steady at ≈2-fold. (*D*) Linear regression analysis comparing systemic levels of human VEGF–VEGF Trap to tumor volume reveals that increasing tumor volume directly correlates with increasing complex levels. ($P < 0.0001$.)

vascular tone in the adult (26). We make the unexpected observation that constitutive VEGF production by normal adult tissues is sufficient to mask the lower levels made by most tumors, making it difficult to use peripheral levels of VEGF as a reliable indicator of tumor burden. However, in mice, VEGF production by tumors is clearly related to tumor size, and, when tumors become quite large, the VEGF Trap complex assays readily detect the tumors' VEGF contribution.

Our observations are consistent with recent studies by Bocci *et al.* (27), which reported that plasma VEGF levels are normally very low or undetectable, but are rapidly increased upon treatment with blocking VEGFR2 antibodies. In these experiments, the observed acute increase in circulating VEGF was not associated with increased VEGF expression in normal tissues, or the tumors, but reflected displacement of VEGF from VEGF receptors. It was also noted that maximal VEGF release occurred at antibody doses that produced near optimal anti-tumor effects, suggesting that maximal VEGF receptor blockade was attained. By extension, the induced increases in plasma VEGF could be used to guide dosing of anti-VEGFR antibodies.

The findings reported by Bocci *et al.* also support the notion that, in normal adult tissues, there is substantial basal production of VEGF, which is locally sequestered and thus not readily measured in the periphery unless it is dislodged. However, measurement of VEGF in the circulation after its displacement by anti-VEGFR antibodies cannot account for VEGF sequestered by binding to sites other than VEGFR1 or VEGFR2 (e.g., neuropilins or heparin) and thus cannot be used to calculate total VEGF production rates in host or tumors. Studies with the VEGF Trap, which also displaces tissue bound VEGF, extend these findings by precisely determining and comparing host and tumor production rates of VEGF. We also show that the observations made in mice seem to also apply to humans and that the levels of VEGF Trap complexed to VEGF can serve as a sensitive guide for the effective dosing of this particular therapeutic candidate. By extension, determination of the dose required to achieve maximum levels of circulating complexes involving a blocker and its target could serve as a useful guide for the dosing of any therapeutic agent that forms long-lived inert circulating complexes with its target.

The sustained circulating levels of VEGF complex observed after VEGF Trap administration is not seen with VEGF-blocking. Unlike the VEGF Trap, which forms an inert 1:1 complex with VEGF that retains the same circulating half-life as unbound VEGF Trap, antibodies to VEGF form heterogeneous multimeric complexes with their antigens, which are cleared much more rapidly than the unbound antibodies. Thus, such immune complexes are not accessible for assays in the systemic circulation, and it is not possible to use systemic levels of such complexes as a guide to VEGF production or to having achieved efficacious antibody levels. Moreover, the formation of such immune complexes could produce undesirable off-mechanism effects. For example, Meyer *et al.* report that bevacizumab forms immune complexes with VEGF that can induce platelet aggregation, which they suggest "might be a possible cause for unexpected arterial thromboembolic events in clinical trials."** In addition, immune complexes can deposit in tissues, including the kidney, potentially contributing to renal damage; consistent with this, we show that VEGF antibodies complexed to VEGF have a much higher propensity to deposit in kidney glomeruli compared with VEGF Trap complexes. Further consistent with this, Gerber *et al.* have reported "anti-VEGF (antibody) depo-

**Meyer, T., Robson, T., Amirkhosravi, A., Langer, F., Desai, H., Amaya, M., Elias, P., Francis, J. L. (2007) *Am. Soc. Hematol.* 108:1091 (abstr.).

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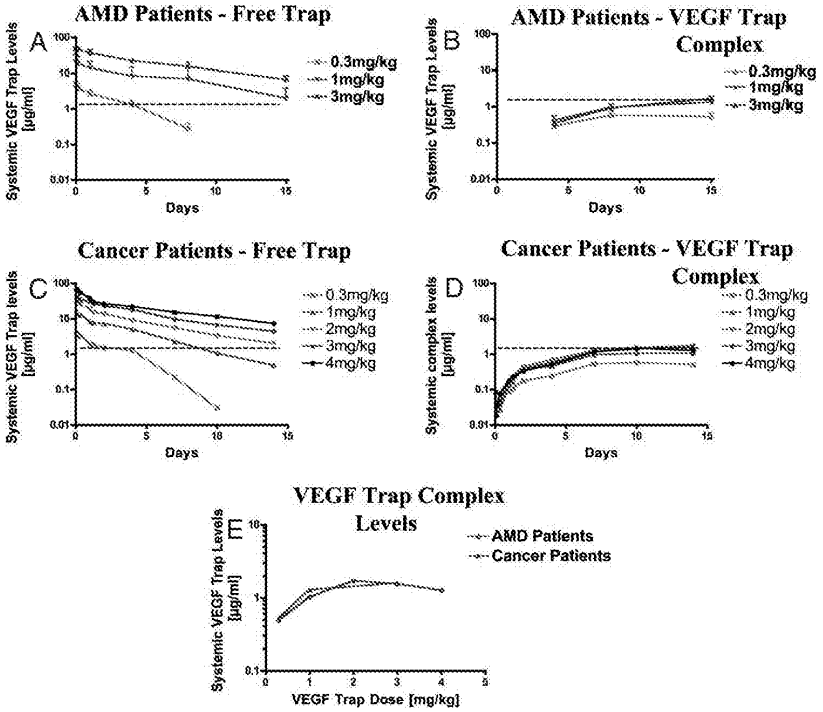


Fig. 6. Circulating free VEGF Trap and human VEGF–VEGF Trap complex levels are very similar in the plasmas of AMD and cancer patients. (*A* and *B*) Patients with AMD received a single i.v. bolus of VEGF Trap at 0.3, 1, or 3 mg/kg, and free VEGF Trap and complex levels were measured at 2 and 4 h and 1, 4, 8, and 15 days (n = 7, 0.3 mg/kg; n = 7, 1 mg/kg; n = 5, 3 mg/kg). (*C* and *D*) Patients with cancer received a single i.v. bolus of VEGF Trap at 0.3, 1, 2, 3, or 4 mg/kg, and free VEGF Trap and complex levels were measured at 1, 2, 4, and 8 h and 1, 2, 4, 7, 10, and 14 days (n = 3, 0.3 mg/kg; n = 7, 1 mg/kg; n = 6, 2 mg/kg; n = 5, 3 mg/kg; n = 7, 4 mg/kg). (*E*) Complex levels in AMD patients at 15 days and cancer patients at 14 days were plotted against the different doses revealing an almost exact overlap. Dotted lines denote the steady-state circulating levels of VEGF–VEGF Trap complex in AMD and cancer patients.

sition in glomeruli" with complement C3 staining and glomerulosclerosis, "which was generally more severe in animals treated with high-affinity mAbs," to VEGF (28). Thus, VEGF Trap, which does not form multimeric immune complexes but instead forms inert 1:1 complexes with VEGF, may not share the same adverse effect profile as anti-VEGF antibodies that can form immune complexes.

In summary, our studies show that assays of free and bound VEGF Trap can serve as useful indicators for the proportion of bioavailable VEGF that is bound and neutralized at a given dose of VEGF Trap. In mice, the majority of endogenous VEGF is captured at doses that result in maximal, steady-state levels of VEGF Trap–VEGF complex, at which point near-optimal efficacy is typically attained. Use of this assay in cancer patients might similarly allow for rapid determination of dosing regimens that are likely to be efficacious. Importantly, application of these assays in early stage clinical trials in patients indicates that the doses currently being evaluated in ongoing clinical studies are in the efficacious range (25, 29–32, ¶).

Materials and Methods

ELISAs. ***Free VEGF Trap and Complex Measurement.*** Levels of free VEGF Trap were measured by using a functional ELISA, which uses $VEGF_{165}$ as the capture and an antibody to the IgG2 domain of VEGFR1 as the report. Mouse VEGF–VEGF Trap complex is measured by using an antibody to mouse VEGF as the capture and the same antibody as above as the report. Human VEGF–VEGF Trap complex is measured by using an antibody to human VEGF as the capture and an antibody to human Fc as the report. ***MALLS Coupled to SEC.*** A multiangle laser light scattering instrument was coupled to a size exclusion column to measure the molar mass and aggregation of $VEGF_{165}$ bound to VEGF Trap or bevacizumab.

Immunocytochemistry of VEGF Trap/VEGF and Bevacizumab–VEGF Complex Deposition in Kidney Glomeruli. Preformed $VEGF_{165}$–VEGF Trap or $VEGF_{165}$–bevacizumab complexes were injected into the left ventricles of C57bl6 mice, and deposition in the kidney was determined immunocytochemically.

Calculation of VEGF Production Rates Based on Steady-State VEGF–VEGF Trap Complex Levels in Mouse and Man. Endogenous VEGF production rates were determined by using the following equation: Complex production rate [μg/day per ml of volume of distribution(ml-d)] = $0.5 \times C_{ss}$ μg/ml per $t_{1/2}$ days = $0.5 \times C_{ss}/t_{1/2}$ μg/ml-d. Because VEGF accounts for 1/4 of the mass of the complex, the VEGF production rate (μg per day per ml of volume of distribution) = $0.25 \times (0.5 \times C_{ss}$ μg/ml per $t_{1/2}$ days) = $0.125 \times C_{ss}/t_{1/2}$ μg/ml-d.

Tumor Implantation. Tumor cell lines were implanted s.c. into the right flank of 7- to 9-week-old male SCID/CB17 mice, and serum samples were taken at termination of the experiment. To assess the relationship between tumor volume and human complex levels, A673 tumors were grown in mice to different sizes, at

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which point the mice were treated with a single bolus of 25 mg/kg VEGF Trap, and tumor volume and mouse and human complex levels measured over a 2-week period.

Human Clinical Trials. Clinical trial design for the studies presented herein are available in refs. 25, 29, and 31–34.

Data Analysis. Linear regression analysis comparing circulating human VEGF–VEGF Trap complex levels with tumor volume was done by using the data analysis package in GraphPad Prism. Pharmacokinetic analyses were done by using the WinNonlin PK/PD modeling and analysis package (Pharsight, Mountain View, CA). Molar masses of proteins and their complexes were determined by using ASTRA software (Wyatt Technology, Santa Barbara, CA) as described in ref. 35.

Additional Details. For a more detailed description of the methods, see *SI Materials and Methods*.

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Intravitreal Aflibercept Injection for Neovascular Age-related Macular Degeneration

Ninety-Six–Week Results of the VIEW Studies

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Purpose: To determine efficacy and safety of intravitreal aflibercept in patients with neovascular age-related macular degeneration (AMD) during a second year of variable dosing after a first-year fixed-dosing period.

Design: Two randomized, double-masked, active-controlled, phase 3 trials.

Participants: Two thousand four hundred fifty-seven patients with neovascular AMD.

Methods: From baseline to week 52, patients received 0.5 mg intravitreal ranibizumab every 4 weeks (Rq4), 2 mg aflibercept every 4 weeks (2q4), 0.5 mg aflibercept every 4 weeks (0.5q4), or 2 mg aflibercept every 8 weeks (2q8) after 3 monthly injections. During weeks 52 through 96, patients received their original dosing assignment using an as-needed regimen with defined retreatment criteria and mandatory dosing at least every 12 weeks.

Main Outcome Measures: Proportion of eyes at week 96 that maintained best-corrected visual acuity (BCVA; lost <15 letters from baseline); change from baseline in BCVA.

Results: Proportions of eyes maintaining BCVA across treatments were 94.4% to 96.1% at week 52 and 91.5% to 92.4% at week 96. Mean BCVA gains were 8.3 to 9.3 letters at week 52 and 6.6 to 7.9 letters at week 96. Proportions of eyes without retinal fluid decreased from week 52 (60.3% to 72.4%) to week 96 (44.6% to 54.4%), and more 2q4 eyes were without fluid at weeks 52 and 96 than Rq4 eyes (difference of 10.4% [95% confidence interval {CI}, 4.9–15.9] and 9.0% [95% CI, 3.0–15.1]). Patients received on average 16.5, 16.0, 16.2, and 11.2 injections over 96 weeks and 4.7, 4.1, 4.6, and 4.2 injections during weeks 52 through 96 in the Rq4, 2q4, 0.5q4, and 2q8 groups, respectively. The number of injections during weeks 52 through 96 was lower in the 2q4 and 2q8 groups versus the Rq4 group (differences of −0.64 [95% CI, −0.89 to −0.40] and −0.55 [95% CI, −0.79 to −0.30]; $P < 0.0001$, post hoc analysis). Incidences of Antiplatelet Trialists' Collaboration–defined arterial thromboembolic events were similar across groups (2.4% to 3.8%) from baseline to week 96.

Conclusions: All aflibercept and ranibizumab groups were equally effective in improving BCVA and preventing BCVA loss at 96 weeks. The 2q8 aflibercept group was similar to ranibizumab in visual acuity outcomes during 96 weeks, but with an average of 5 fewer injections. Small losses at 96 weeks in the visual and anatomic gains seen at 52 weeks in all arms were in the range of losses commonly observed with variable dosing. *Ophthalmology 2014;121:193-201 © 2014 by the American Academy of Ophthalmology.*



The introduction of antiangiogenic therapy to treat neovascular age-related macular degeneration (AMD) has vastly changed common paradigms in this important entity usually referred to as "the leading cause of legal blindness in the developed world."[1] The prospective, masked, randomized, pivotal trials for ranibizumab, called the Anti-Vascular Endothelial Growth Factor (VEGF) Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in AMD (ANCHOR) and the Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD (MARINA), showed clear superiority of monthly intravitreal ranibizumab administration compared with sham or with the previous gold standard, photodynamic therapy.[2,3] After approval in 2006 to treat neovascular AMD, intravitreal ranibizumab was embraced quickly by the ophthalmic

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community. Odds for visual acuity loss resulting from neovascular AMD markedly decreased with fixed monthly ranibizumab therapy.[4] Thus, this monthly treatment regimen was included in the ranibizumab Food and Drug Administration label.

Although the visual results in the clinical trials were excellent with the monthly dosing regimens, in clinical practice, the repetitive office visits and injections represent an overwhelming management challenge for patients and their families. Evaluations of actual treatment patterns revealed that most patients were examined and treated far less frequently than recommended by the results of the studies, leading to inferior outcomes.[5,6] Undertreatment prevents patients from optimally benefitting from one of the major therapeutic breakthroughs in ophthalmology.

To reduce the treatment burden and still conform to a structured regimen, treatment intervals were expanded in studies such as the Phase IIIB, Multicenter, Randomized, Double-Masked, Sham Injection-Controlled Study of the Efficacy and Safety of Ranibizumab in Patients with AMD-Related Subfoveal Choroidal Neovascularization (CNV), with or without Classic CNV(PIER), and the Efficacy and Safety of Monthly versus Quarterly Ranibizumab Treatment in Neovascular Age-Related Macular Degeneration (EXCITE).[7,8] However, the visual acuity benefit of ranibizumab therapy was reduced markedly when treatment intervals were increased up to 3 months. It was recognized that treatment of recurrence had to take place in a timely manner to prevent functional loss.

Pro re nata (PRN) treatment, or treatment as needed, was evaluated first in the Prospective Optical Coherence Tomography Imaging of Patients with Neovascular AMD Treated with Intra-Ocular Ranibizumab (PRONTO) study, a small, single-center, carefully monitored investigator-driven trial.[9] Physicians used an optical coherence tomography (OCT)-guided variable-dosing regimen with intravitreal ranibizumab and achieved outcomes comparable with those observed in the phase 3 clinical studies, which used a fixed monthly monitoring and dosing regimen. In contrast, the Open-Label Extension Trial of Ranibizumab for Choroidal Neovascularization Secondary to Age-Related Macular Degeneration (HORIZON) trial, a PRN extension trial after monthly ranibizumab for 2 years, reported that the initial benefit achieved by 2 years of monthly retreatment was lost progressively when switching to a PRN treatment paradigm.[10]

The Comparison of Age-Related Macular Degeneration Treatments Trials (CATT) group subsequently designed a large, multicenter, prospective, randomized trial that compared a fixed monthly regimen with a flexible as-needed regimen using the 2 most commonly used anti-VEGF therapies, ranibizumab and bevacizumab.[11] Unlike the PRONTO study,[9] the indication for retreatment in the CATT study was focused strictly on the presence of fluid on OCT, rather than on overall retinal thickness changes; injection was indicated whenever intraretinal, subretinal, or sub–retinal pigment epithelium fluid was identified during monthly OCT monitoring. Although the primary outcome showed noninferiority between ranibizumab and bevacizumab when administered according to similar regimens, the visual acuity gains and morphologic improvement were greater for the monthly groups as compared with the as-needed groups, especially in year 2.[11] To achieve these results, the total number of injections in the as-needed ranibizumab and bevacizumab arms was high: 6.9 and 7.7 injections over the first year and 12.6 and 14.1 injections over 2 years, respectively, with monthly monitoring visits. It is also important to note that the bevacizumab as-needed group did not meet the noninferiority criteria with an as-needed dosing schedule.[12]

Intravitreal aflibercept, a fusion protein of key domains from human VEGF receptors 1 and 2 with the constant region (Fc) of human immunoglobulin G, recently was approved for the treatment of neovascular AMD.[13] As a designed molecule featuring optimal pharmacologic characteristics to inhibit intraocular VEGF, intravitreal aflibercept injection offers improved binding affinity and superior pharmacokinetics in an iso-osmotic formulation.[14,15]

The VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD (VIEW 1 and 2) studies were the largest controlled trials of anti-VEGF agents in AMD ever performed, recruiting more than 2400 patients with treatment-naïve neovascular AMD from more than 360 centers worldwide.[16] The focus of the trials was to compare the standard of care (ranibizumab 0.5 mg at monthly intervals) with 2 doses (2 and 0.5 mg) of intravitreal aflibercept and 2 regimens (monthly and every 2 months after 3 initial monthly doses). All intravitreal aflibercept groups were clinically equivalent to monthly ranibizumab in maintaining visual acuity at week 52.[16] This result also was true when drug was administered every 2 months, which allowed a substantially reduced monitoring and treatment frequency, and thus introduced a novel treatment strategy to manage neovascular AMD.[16]

After the 52-week primary end point, a follow-up phase of the VIEW trials, up to 96 weeks, was based on a protocol that required a switch of all regimens from the fixed monthly or every 2 months regimen to a variable regimen requiring at least quarterly dosing (capped PRN); interim injections were allowed based on an assessment of anatomic and visual parameters. The aim of the current study was to investigate the safety and efficacy of an extended treatment interval after 1 year of rigorously scheduled fixed treatments. The 96-week data for the integrated VIEW studies describing characteristics and outcomes of a variable dosing regimen are presented and discussed in this article.

Methods

Design

The VIEW 1 and 2 studies were 2 similarly designed randomized, double-masked, active-controlled, parallel-group, multicenter, 96-week phase 3 trials comparing the efficacy and safety of intravitreal aflibercept and ranibizumab in patients with neovascular AMD.[16] The VIEW 1 study was carried out from July 2007 through July 2011 in the United States and Canada, and the VIEW 2 study was carried out from April 2008 through August 2011 in Europe, the Middle East, the Asia-Pacific region, and

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Latin America. Patients were screened and/or randomized at 362 sites in the VIEW studies. Each institutional review board or ethics committee approved the study protocols. Both trials were registered with ClinicalTrials.gov (identifier nos. NCT00509795 and NCT00637377), and all patients signed a written consent form before initiation of the study-specific procedures. The VIEW 1 and 2 studies were conducted in compliance with regulations of the Health Insurance Portability and Accountability Act and the tenets of the Declaration of Helsinki.

The design of VIEW studies has been described previously.[16] In brief, patients 50 years of age and older with active, subfoveal, CNV lesions (or juxtafoveal lesions with leakage affecting the fovea) secondary to neovascular AMD were eligible for enrollment if CNV made up at least 50% of total lesion size and BCVA was between 25 and 73 Early Treatment Diabetic Retinopathy Study (ETDRS) letters (20/320–20/40 Snellen equivalent). Only 1 eye from each patient was included in the study. Patients were randomized in a 1:1:1:1 ratio to receive 1 of the following 4 regimens in the study eye for the first 52 weeks: (1) 0.5 mg intravitreal ranibizumab every 4 weeks (Rq4), (2) 2 mg intravitreal aflibercept every 4 weeks (2q4), (3) 0.5 mg intravitreal aflibercept every 4 weeks (0.5q4), and (4) 2 mg intravitreal aflibercept every 8 weeks (2q8) after 3 initial monthly injections. During the follow-up period from weeks 52 to 96, patients continued to receive the same dose of study drugs as in the first 52 weeks, but received injections at least every 12 weeks, with monthly evaluations for interim injections based on prespecified retreatment criteria (mandatory quarterly dosing with examination-guided interim injections or capped-PRN). Criteria for retreatment were new or persistent fluid on OCT, an increase in central retinal thickness of 100 μm or more compared with the lowest previous value, loss of 5 ETDRS letters or more from the best previous score in conjunction with recurrent fluid on OCT, new-onset classic neovascularization, new or persistent leak on fluorescein angiography, new macular hemorrhage, or a time lapse of 12 weeks since the previous injection.

Outcome Measures

The primary efficacy end point of the VIEW 1 and VIEW 2 studies was noninferiority of the intravitreal aflibercept regimens to ranibizumab in the proportion of patients maintaining visual acuity (losing <15 ETDRS letters) at week 52.[16] Prespecified secondary efficacy end points compared the change among treatment groups in visual acuity and anatomic outcomes from baseline to week 52.[16] Prespecified primary and secondary efficacy outcomes of the VIEW 1 and VIEW 2 studies at week 52 have been reported previously.[16] Efficacy end points evaluated after week 52 all were exploratory and included the proportion of patients maintaining visual acuity (losing <15 ETDRS letters), the mean change in BCVA from baseline, the proportion of patients gaining 15 letters or more, mean change from baseline CNV size, and the proportion of patients without retinal fluid at week 96. The mean change in central retinal thickness also was determined from baseline through week 96. Additional end points during the exploratory follow-up phase were the number of study drug injections and the proportion of patients receiving fewer than 6 injections and 6 injections or more between weeks 52 and 96.

Patients were evaluated for BCVA at screening, at the day of treatment initiation, and every 4 weeks thereafter through week 96, as well as 1 week after the first treatment for safety reasons. In the VIEW 1 study, OCT was performed at screening, at the day of treatment initiation, and at weeks 4, 12, 24, 36, and 52 and every 4 weeks thereafter through week 96. In the VIEW 2 study, OCT was performed at every visit. The OCT images were obtained with a time-domain Stratus instrument (Carl Zeiss Meditec, Dublin, CA) and was evaluated by an independent central reading center (VIEW 1, Duke Reading Center, Durham, NC; VIEW 2, Vienna Reading Center, Vienna, Austria). Fundus photography and fluorescein angiography were performed at screening and at weeks 24, 52, 72, and 96, and the results were evaluated by an independent central reading center (Digital Angiography Reading Center, New York, NY). Areas of visible active CNV (classic, occult, or both) were identified when angiographic analyses showed evidence of visible neovascular tissue accompanied by late leakage or pooling of dye.

Statistical Analysis

Data from the VIEW 1 and VIEW 2 studies were pooled for the purpose of presentation in this report. The proportion of patients maintaining visual acuity (losing <15 ETDRS letters) at week 52 was analyzed in the per-protocol set as defined previously.[16] The proportion of patients maintaining visual acuity (losing <15 ETDRS letters) at week 96 was analyzed in the full analysis set, which included all randomized patients who received any study medication and had a baseline BCVA measurement and at least 1 BCVA assessment after baseline. All other visual and anatomic end points were analyzed in the full analysis set. The last-observation-carried-forward approach was used to impute missing data. Safety end points at weeks 52 and 96 were analyzed in the safety analysis set, which included all patients who received any study medication. Treatment experience over the 2 years of study was analyzed in the safety analysis set. Treatment experience in the second year was analyzed in patients who completed study treatments. Between-group differences in the number of injections from weeks 52 to 96 were analyzed with an analysis of variance in a post hoc analysis.

Results

Patient Disposition and Baseline Characteristics

The VIEW 1 and 2 studies randomized a total of 2457 patients; 2419 (98.5%) patients received at least 1 dose of study medication, and 2245 (91.4%) patients completed 52 weeks of study. A total of 2235 (91.0%) patients entered the second year, and 2063 (84.0%) patients completed 96 weeks of study. The percentage of patients completing the study was similar among treatment groups at both weeks 52 and 96 (Table 1, available at http://aaojournal.org). Reasons for discontinuation before week 96 included consent withdrawal occurring in 5.0% to 6.5% of patients and adverse events occurring in 2.6% to 4.9% of patients across treatment groups (Table 1, available at http://aaojournal.org). Baseline demographics and disease characteristics were evenly balanced among all treatment groups (Table 2).

Efficacy

The proportion of patients maintaining visual acuity ranged from 94.4% to 96.1% at week 52 (Fig 1A). Both monthly and every 2 months intravitreal aflibercept regimens were statistically noninferior (with a margin within 5%) to monthly ranibizumab at week 52 (mean of Rq4 minus intravitreal aflibercept, −0.9% [95% confidence interval (CI), −3.5 to 1.7] for 2q4; −1.7% [95% CI, −4.2 to 0.9] for 0.5q4; and −0.9% [95% CI, −3.5 to 1.7] for 2q8). Largely similar proportions of patients (91.5% to 92.4%) maintained visual acuity across all treatment groups at week 96 (Fig 1A). The mean increase in BCVA from baseline was largely similar among treatment groups throughout the 96 weeks of the study (Fig 1B). At week 96, the mean BCVA gains were 7.9 letters, 7.6 letters, 6.6 letters, and 7.6 letters in the Rq4,

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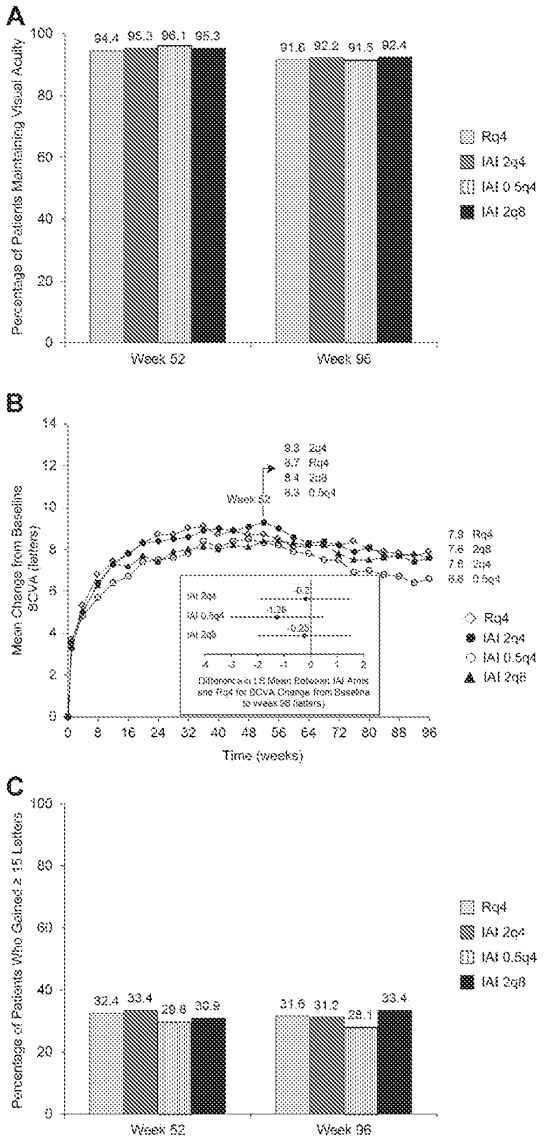


Figure 1. Graphs showing visual acuity outcomes in the total study cohort. **A**, Proportion of patients maintaining visual acuity (losing <15 Early Treatment Diabetic Retinopathy Study letters). Per-protocol and full analysis sets were used for weeks 52 and 96, respectively. At week 52, n = 538, n = 559, n = 538, and n = 535 for Rq4, 2q4, 0.5q4, and 2q8, respectively. At week 96, n = 595, n = 613, n = 597, and n = 607 for Rq4, 2q4, 0.5q4, and 2q8, respectively. **B**, Mean change from baseline best-corrected visual acuity (BCVA). The inset shows the difference in least square (LS) mean (with 95% confidence interval) between intravitreal aflibercept arms and ranibizumab (aflibercept minus ranibizumab) for BCVA change from baseline to week 96, full analysis set. **C**, Proportion of patients who gained 15 letters or more, full analysis set. At weeks 52 and 96, n = 595, n = 613, n = 597, and n = 607 for Rq4, 2q4, 0.5q4, and 2q8, respectively. Missing values were imputed using the last-observation-carried-forward method in (**A**), (**B**), and (**C**). The outcomes for the aflibercept and ranibizumab groups were similar in (**A**), (**B**), and (**C**) at both weeks 52 and 96. IAI = intravitreal aflibercept injection; Rq4 = 0.5-mg intravitreal ranibizumab every 4 weeks; 2q4 = 2 mg every 4 weeks; 0.5q4 = 0.5 mg every 4 weeks; 2q8 = 2 mg every 8 weeks after 3 initial monthly injections.

2q4, 0.5q4, and 2q8 groups, respectively; these gains represented a 1- to 2-letter loss in all groups during the capped PRN (modified quarterly dosing) phase, compared with the gains observed at week 52 (8.7, 9.3, 8.3, and 8.4 letters, respectively). Overall, 29.8% to 33.4% of patients in all treatment groups gained 15 letters or more from baseline to week 52 (Fig 1C). The proportions of patients who gained 15 letters or more from baseline to week 96 were similar and ranged from 28.1% to 33.4% (Fig 1C). Across treatment groups, largely similar proportions of patients had a BCVA of 20/40 or better or had an improvement from baseline BCVA of 0 letters or more, 10 letters or more, and 30 letters or more at weeks 52 and 96 (Table 3, available at http://aaojournal.org).

During the capped PRN phase (requiring at least quarterly dosing), there was a minor loss in the anatomic improvements that had been seen at week 52. At week 96, patients had an average increase in central retinal thickness of 10 μm, 10 μm, 10 μm, and 6 μm from week 52 in the Rq4, 2q4, 0.5q4, and 2q8 groups, respectively (Fig 2A). The proportion of patients with no retinal fluid on time-domain OCT (observed cases) ranged from 60.3% to 72.4% at week 52, with higher percentages of 2q4 and 2q8 patients having no retinal fluid compared with Rq4 patients (mean of aflibercept minus Rq4, 10.4% [95% CI, 4.9−15.9] for 2q4 and 5.7% [95% CI, 0−11.4] for 2q8). The percentage of patients with no retinal fluid decreased from week 52 to week 96 in all treatment groups. Nevertheless, a higher percentage of 2q4 patients had no retinal fluid at week 96 compared with Rq4 patients (mean of 2q4 minus Rq4, 9.0% [95% CI, 3.0−15.1]; Fig 2B). In contrast, the mean decreases in CNV area were maintained from week 52 (range, 3.9−5.3 mm^2) to week 96 (range, 3.7−5.1 mm^2) in all treatment groups. A lower CNV area was observed at week 52 for 2q4 in comparison with Rq4 (least squares mean of 2q4 minus Rq4, −0.74 mm^2 [95% CI, −1.27 to −0.21]), but was not maintained at week 96.

Number of Injections

The mean number of injections from week 0 to week 96 was 16.5 (standard deviation [SD], 3.7), 16.0 (SD, 3.2), 16.2 (SD, 4.0), and 11.2 (SD, 2.9) in the Rq4, 2q4, 0.5q4, and 2q8 groups, respectively. The mean number of injections from week 52 to week 96 was 4.7 (SD, 2.2), 4.1 (SD, 1.8), 4.6 (SD, 2.2), and 4.2 (SD, 1.7) in the Rq4, 2q4, 0.5q4, and 2q8 groups, respectively. In a post hoc analysis, this number of injections from week 52 to week 96 was lower in the 2q4 and 2q8 groups versus the Rq4 group: mean of aflibercept minus Rq4, −0.64 (95% CI, −0.89 to −0.40) for 2q4 and −0.55 (95% CI, −0.79 to −0.30) for 2q8 ($P < 0.0001$ for both). The proportion of patients who received fewer than 6 injections and 6 injections or more during weeks 52 to 96 are shown in Figure 3A. Overall, higher percentages of 2q4 and 2q8 patients received fewer than 6 injections compared with Rq4 patients, whereas a higher percentage of Rq4 patients received 6 injections or more compared with 2q4 and 2q8 patients (Fig 3A, B).

Safety

Safety profiles of both intravitreal aflibercept and ranibizumab were favorable. Ocular adverse events occurring in 10% or more of

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Table 2. Patient Demographics and Baseline Characteristics, Full Analysis Set

	0.5 mg Intravitreal Ranibizumab Every 4 Weeks (n = 595)	Intravitreal Aflibercept Injection 2 mg Every 4 Weeks (n = 613)	Intravitreal Aflibercept Injection 0.5 mg Every 4 Weeks (n = 597)	Intravitreal Aflibercept Injection 2 mg Every 8 Weeks after 3 Initial Monthly Injections (n = 607)
Female, n (%)	341 (57.3)	370 (60.4)	314 (52.6)	353 (58.2)
Race, n (%)				
White	509 (85.5)	521 (85.0)	510 (85.4)	504 (83.0)
Asian	60 (10.1)	70 (11.4)	66 (11.1)	73 (12.0)
Other*	26 (4.4)	22 (3.6)	21 (3.5)	30 (5.0)
Age (SD), yrs	75.6 (8.7)	75.9 (8.4)	76.5 (8.5)	75.8 (8.8)
BCVA (SD), letters	53.9 (13.4)	54.0 (13.6)	53.6 (13.8)	53.6 (13.5)
Central retinal thickness (SD), μm	296 (123)†	299 (126)‡	296 (132)†	306 (134)§
Area of CNV (SD), mm^2	7.1 (5.3)‖	7.4 (5.5)¶	7.1 (4.9)#	7.2 (5.4)**
Type of CNV, n (%)				
Minimally classic	205 (34.5)	217 (35.4)	200 (33.5)	216 (35.6)
Occult	231 (38.8)	233 (38.0)	234 (39.2)	228 (37.6)
Predominantly classic	152 (25.5)	159 (25.9)	161 (27.0)	159 (26.2)
Missing	7 (1.2)	4 (0.7)	2 (0.3)	4 (0.7)
Total lesion size (SD), mm^2	7.5 (5.6)‖	7.9 (5.8)¶	7.5 (5.2)#	7.6 (5.6)**
Total NEI VFQ score (SD)	72.4 (18.1)†	70.3 (18.1)††	72.6 (18.0)‡‡	70.4 (18.0)§§

BCVA = best-corrected visual acuity; CNV = choroidal neovascularization; NEI VFQ = National Eye Institute Visual Function Questionnaire; SD = standard deviation.
*Included American Indian or Alaska Native, Black or African American, Native Hawaiian or other Pacific Islander, multiracial patients, and those who did not report their race.
†n = 594.
‡n = 611.
§n = 603.
‖n = 589.
¶n = 610.
#n = 596.
**n = 605.
††n = 609.
‡‡n = 592.
§§n = 599.

patients across treatment groups were conjunctival hemorrhage (range, 21.7%–28.1%) and eye pain (range, 7.0%–10.8%) from baseline to week 52, and conjunctival hemorrhage (range, 23.7%–29.9%), retinal hemorrhage (range, 13.6%–16.2%), reduced visual acuity (range, 11.3%–13.0%), eye pain (range, 8.9%–12.1%), vitreous detachment (range, 7.7%–10.0%), and increased intraocular pressure (range, 6.2%–10.8%) from baseline to week 96. Any intraocular inflammatory response (predefined adverse event of interest) was reported in 0.8%, 0.7%, 0.3%, and 0.2% of patients from baseline to week 52 and in 1.5%, 1.1%, 0.8%, and 0.5% of patients from baseline to week 96 in the Rq4, 2q4, 0.5q4, and 2q8 groups, respectively. Serious ocular adverse events were infrequent and occurred with a similar rate across all treatment groups (Table 4). Major serious systemic adverse events were fall and pneumonia from baseline to week 52, and fall, pneumonia, atrial fibrillation, and myocardial infarction from baseline to week 96 (Table 5, available at http://aaojournal.org). In general, serious systemic adverse events were typical of those reported in this population of elderly patients who receive intravitreal treatment for neovascular AMD. The incidence of arterial thromboembolic events as defined by the Antiplatelet Trialists' Collaboration criteria was similar among treatment groups from both baseline to week 52 and from baseline to week 96 (Table 6). The percentage of deaths was 1.2%, 0.7%, 0.5%, and 1.5% from baseline to week 52 and 2.7%, 2.1%, 3.2%, and 3.3% from baseline to week 96 in the Rq4, 2q4, 0.5q4, and 2q8 groups, respectively. The incidences and patterns of deaths were not different among treatment groups.

Discussion

The results from the follow-up regimen of mandatory quarterly dosing with intervening as-needed injections (capped PRN) in the second year of the VIEW studies confirm the sustained improvements in visual acuity, central retinal thickness, and CNV size achieved by fixed dosing regimens of intravitreal aflibercept and ranibizumab during the first year.[16] All intravitreal aflibercept regimens were as effective as ranibizumab in increasing visual acuity and reducing retinal thickness and CNV size over 2 years of the VIEW studies. Small decreases in visual and anatomic improvements from week 52 to 96 were observed in all treatment groups, similar to declines seen in other randomized clinical trials when switching to treatment regimens with a variable component.[11] Of note was a decrease in the proportion of patients with no retinal fluid from week 52 to 96 after switching to a more variable dosing regimen in all treatment groups. Nevertheless, more patients in the 2q4 group had no

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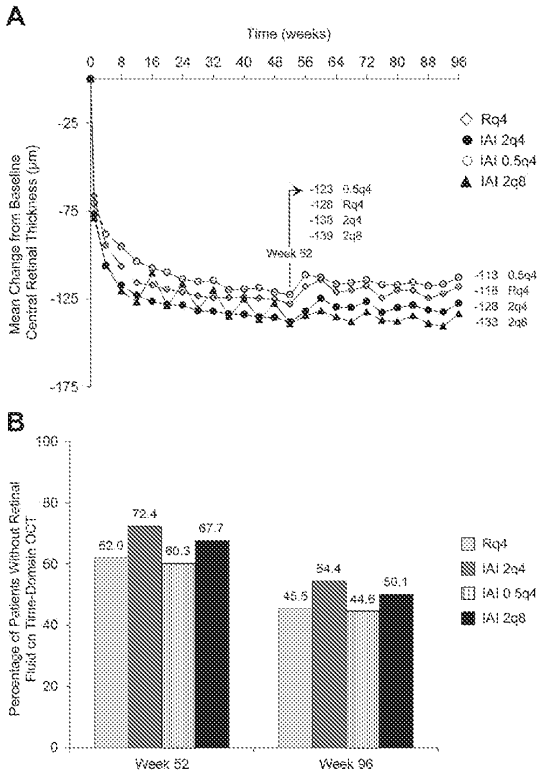


Figure 2. Graphs showing anatomic outcomes in total study cohort. **A**, Mean change from baseline central retinal thickness, full analysis set. Missing values were imputed using the last-observation-carried-forward method. The outcomes for the aflibercept and ranibizumab groups were similar at both weeks 52 and 96. **B**, Proportion of patients without fluid on time-domain optical coherence tomography (OCT) images. Observed values in full analysis set. Number of patients included in the Rq4, 2q4, 0.5q4, and 2q8 groups were 537, 558, 527, and 539 at week 52, and 508, 522, 493, and 505 at week 96, respectively. IAI = intravitreal aflibercept injection; Rq4 = 0.5-mg intravitreal ranibizumab every 4 weeks; 2q4 = 2 mg every 4 weeks; 0.5q4 = 0.5 mg every 4 weeks; 2q8 = 2 mg every 8 weeks after 3 initial monthly injections.

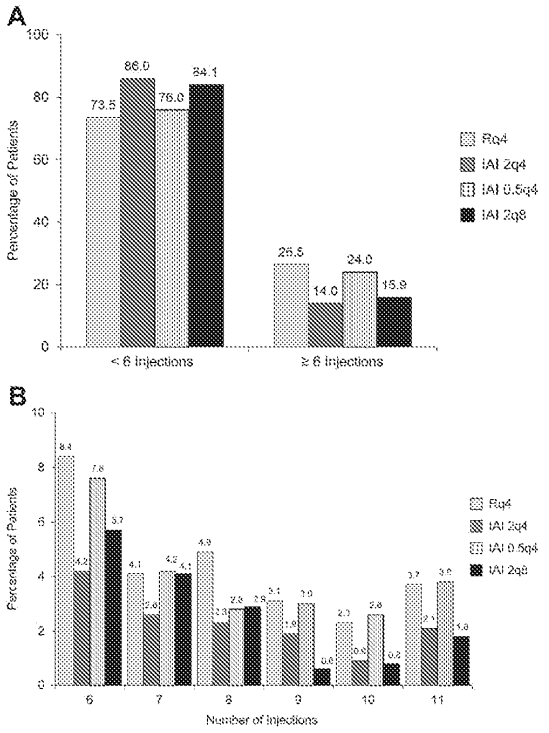


Figure 3. Graphs showing proportion of patients by the number of injections during weeks 52 to 96. **A**, Percentages of patients who received fewer than 6 injections and 6 or more injections. **B**, Percentage of patients who received 6 to 11 injections. The maximum number of injections was 11 in all treatment groups. Patients who completed the second year follow-up phase medications were included in the analyses shown in (**A**) and (**B**). Number of patients included in the Rq4, 2q4, 0.5q4, and 2q8 groups were 513, 529, 499, and 511, respectively, in (**A**) and (**B**). IAI = intravitreal aflibercept injection; Rq4 = 0.5-mg intravitreal ranibizumab every 4 weeks; 2q4 = 2 mg every 4 weeks; 0.5q4 = 0.5 mg every 4 weeks; 2q8 = 2 mg every 8 weeks after 3 initial monthly injections.

retinal fluid at week 96, as did both the 2q4 and 2q8 groups at week 52, compared with the Rq4 group. Subtle decreases in the visual and anatomic improvements from week 52 to 96 are likely the result of the variable dosing regimen used. A fixed dosing regimen may provide predictable visual and anatomic outcomes and may mitigate loss of visual and anatomic improvements.

Patients in the 2q8 group achieved visual and anatomic improvements similar to those in the Rq4 and 2q4 groups, but with a mean of 5 fewer injections over 2 years. The significantly fewer average number of injections (post hoc analysis) in the follow-up phase in both 2q4 and 2q8 groups compared with the Rq4 group was driven by more patients in the Rq4 arm receiving the most intense therapy (≥6 injections; 14.0% and 15.9% vs. 26.5%, respectively). These findings suggest that patients with greater disease activity may require fewer injections using intravitreal aflibercept.

Over the 2 years of treatment, a generally favorable safety profile was observed for both intravitreal aflibercept and ranibizumab. No unexpected safety signals were observed with intravitreal aflibercept. The incidence of ocular treatment-emergent adverse events was balanced across all treatment groups, with the most frequent events associated with the injection procedure, the underlying disease, the aging process, or a combination thereof. The incidences of arterial thromboembolic events and death were similar across all treatment groups.

At the time the VIEW studies were designed, the efficacy of variable regimens of anti-VEGF agents was being evaluated as a recommended standard of care in several studies. Current clinical evidence shows that variable regimens, which are unpredictable and require monthly monitoring, are less effective to maintain visual and anatomic improvements gained by fixed dosing regimens. Debate

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Table 4. Serious Ocular Adverse Events in the Study Eye Occurring in More Than 1 Patient in Any Treatment Group, Safety Analysis Set

Serious Adverse Event	Baseline to Week 52				Baseline to Week 96			
	0.5 mg Intravitreal Ranibizumab Every 4 Weeks (n = 595)	*2 mg Intravitreal Aflibercept Injection Every 4 Weeks (n = 613)*	*0.5 mg Intravitreal Aflibercept Injection Every 4 Weeks (n = 601)*	*2 mg Intravitreal Aflibercept Injection Every 8 Weeks after 3 Initial Monthly Injections (n = 610)*	*0.5 mg Intravitreal Ranibizumab Every 4 Weeks (n = 595)*	*2 mg Intravitreal Aflibercept Injection Every 4 Weeks (n = 613)*	*0.5 mg Intravitreal Aflibercept Injection Every 4 Weeks (n = 601)*	*2 mg Intravitreal Aflibercept Injection Every 8 Weeks after 3 Initial Monthly Injections (n = 610)*
Total patients with at least 1 ocular SAE, n (%)	19 (3.2)	13 (2.1)	11 (1.8)	12 (2.0)	26 (4.4)	22 (3.6)	19 (3.2)	24 (3.9)
Macular hole	0	0	2 (0.3)	0	0	0	2 (0.3)	0
Posterior capsule opacification	2 (0.3)	0	0	0	2 (0.3)	0	0	0
Retinal detachment	1 (0.2)	0	2 (0.3)	0	3 (0.5)	1 (0.2)	2 (0.3)	0
Retinal hemorrhage	3 (0.5)	2 (0.3)	1 (0.2)	3 (0.5)	4 (0.7)	3 (0.5)	5 (0.8)	5 (0.8)
Retinal pigment epithelial tear	1 (0.2)	0	1 (0.2)	2 (0.3)	1 (0.2)	0	1 (0.2)	3 (0.5)
Reduced visual acuity	3 (0.5)	2 (0.3)	3 (0.5)	5 (0.8)	5 (0.8)	4 (0.7)	3 (0.5)	7 (1.1)
Endophthalmitis	3 (0.5)	3 (0.5)	0	0	5 (0.8)	4 (0.7)	1 (0.2)	0
Cataract	1 (0.2)	1 (0.2)	1 (0.2)	1 (0.2)	1 (0.2)	4 (0.7)	3 (0.5)	4 (0.7)
Macular degeneration	0	0	0	1 (0.2)	0	0	0	2 (0.3)
Increased intraocular pressure	1 (0.2)	0	1 (0.2)	1 (0.2)	1 (0.2)	0	1 (0.2)	2 (0.3)

SAE = serious adverse event.

continues as to whether the losses in visual acuity are offset by the reductions in treatment and monitoring burden, especially if monitoring is not maintained at the monthly frequency mandated in our study and in the CATT study. Proponents of the treat-and-extend regimen, a treatment strategy involving gradual extension of the treatment and monitoring intervals after initially treating monthly until the macula is dry, suggest that this regimen may result in visual acuity outcomes similar to those seen with monthly therapy, but this has not been demonstrated in a large, randomized

Table 6. Antiplatelet Trialists' Collaboration-Defined Arterial Thromboembolic Events, Safety Analysis Set

	Baseline to Week 52					Baseline to Week 96				
	0.5 mg Intravitreal Ranibizumab Every 4 Weeks (n = 595)	*2 mg Intravitreal Aflibercept Injection Every 4 Weeks (n = 613)*	*0.5 mg Intravitreal Aflibercept Injection Every 4 Weeks (n = 601)*	*2 mg Intravitreal Aflibercept Injection Every 8 Weeks after 3 Initial Monthly Injections (n = 610)*	*All Intravitreal Aflibercept Injections (n = 1824)*	*0.5 mg Intravitreal Ranibizumab Every 4 Weeks (n = 595)*	*2 mg Intravitreal Aflibercept Injection Every 4 Weeks (n = 613)*	*0.5 mg Intravitreal Aflibercept Injection Every 4 Weeks (n = 601)*	*2 mg Intravitreal Aflibercept Injection Every 8 Weeks after 3 Initial Monthly Injections (n = 610)*	*All Intravitreal Aflibercept Injections (n = 1824)*
Any APTC event, n (%)	9 (1.5)	6 (1.0)	12 (2.0)	14 (2.3)	32 (1.8)	19 (3.2)	15 (2.4)	23 (3.8)	22 (3.6)	60 (3.3)
Nonfatal MI	6 (1.0)	3 (0.5)	6 (1.0)	6 (1.0)	15 (0.8)	12 (2.0)	6 (1.0)	12 (2.0)	7 (1.1)	25 (1.4)
Nonfatal stroke	1 (0.2)	2 (0.3)	3 (0.5)	3 (0.5)	8 (0.4)	5 (0.8)	5 (0.8)	3 (0.5)	5 (0.8)	13 (0.7)
Vascular death	2 (0.3)	1 (0.2)	3 (0.5)	5 (0.8)	9 (0.5)	3 (0.5)	5 (0.8)	8 (1.3)	11 (1.8)	24 (1.3)

APTC = Antiplatelet Trialists' Collaboration; MI = myocardial infarction.

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clinical trial.[17,18] The 1-year outcomes of the VIEW studies demonstrate that the average patient can obtain results clinically equivalent to monthly ranibizumab with 2 mg intravitreal aflibercept administered every 8 weeks after 3 initial monthly injections.[16] It is conceivable that a continuation of the every-2-months fixed-dosing regimen using intravitreal aflibercept into the second year would have maintained more effectively the visual and anatomic improvements achieved during the first year. Such a fixed-dosing regimen thus would allow for better outcomes with a substantially lower number of monitoring visits. In addition, a fixed, every-2-months dosing regimen with aflibercept (requiring 5 injections) would approximate the 4.2 injections given with the capped PRN (modified quarterly dosing) regimen in the second year of the VIEW studies. Future studies may shed additional light on the benefit of continuing with an every-2-months fixed-dosing regimen instead of using variable dosing regimens.

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REVIEW

Aflibercept in wet AMD: specific role and optimal use

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Background: Vascular endothelial growth factor (VEGF) is a naturally occurring glycoprotein in the body that acts as a growth factor for endothelial cells. It regulates angiogenesis, enhances vascular permeability, and plays a major role in wet age-related macular degeneration. The consistent association between choroidal neovascularization and increased VEGF expression provides a strong reason for exploring the therapeutic potential of anti-VEGF agents in the treatment of this disorder. Blockade of VEGF activity is currently the most effective strategy for arresting choroidal angiogenesis and reducing vascular permeability, which is frequently the main cause of visual acuity deterioration. In recent years, a number of other molecules have been developed to increase the efficacy and to prolong the durability of the anti-VEGF effect. Aflibercept (EYLEA®; Regeneron Pharmaceutical Inc and Bayer), also named VEGF Trap-eye, is the most recent member of the anti-VEGF armamentarium that was approved by the US Food and Drug Administration in November 2011. Because of its high binding affinity and long duration of action, this drug is considered to be a promising clinically proven anti-VEGF agent for the treatment of wet maculopathy.

Objective: This article reviews the current literature and clinical trial data regarding the efficacy and the pharmacological properties of VEGF-Trap eye and describes the possible advantages of its use over the currently used "older" anti-VEGF drugs.

Methods: For this review, a search of PubMed from January 1989 to May 2013 was performed using the following terms (or combination of terms): vascular endothelial growth factors, VEGF, age-related macular degeneration, VEGF-Trap eye in wet AMD, VEGF-Trap eye in diabetic retinopathy, VEGF-Trap eye in retinal vein occlusions, aflibercept. Studies were limited to those published in English.

Results and conclusion: Two Phase III clinical trials, VEGF Trap-eye Investigation of Efficacy and Safety in Wet AMD (VIEW) 1 and 2, comparing VEGF Trap-eye to ranibizumab demonstrated the noninferiority of this novel compound. The clinical equivalence of this compound against ranibizumab is maintained even when the injections are administered at 8-week intervals, which indicates the potential to reduce the risk of monthly intravitreal injections and the burden of monthly monitoring.

Keywords: aflibercept, AMD, neovascularization, VEGF, VEGF inhibition, VEGF-Trap eye

Introduction

The neovascular form of age-related macular degeneration (AMD), also known as wet AMD, is characterized by the formation of subretinal choroidal neovascularization (CNV) and is the cause of most cases of blindness in the elderly. Wet AMD is the major cause of severe vision loss in developed nations and is estimated to affect >2.5 million people worldwide.[1,2] The patients affected by exudative AMD often experience rapid

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loss of fine resolution central vision over several months, and early visual stabilization is a key issue in preserving visual acuity.[3]

Vascular endothelial growth factor (VEGF) is a naturally occurring glycoprotein in the body that acts as a growth factor selective for endothelial cells. It regulates angiogenesis, enhances vascular permeability, and plays a leading role in wet AMD. The consistent association between CNV and increased VEGF expression provides a strong reason for exploring the therapeutic potential of anti-VEGF agents for the treatment of this disorder.[4] Blockade of VEGF actions is currently the most effective strategy in arresting choroidal angiogenesis and reducing vascular permeability, which is frequently the main cause of visual acuity deterioration.[5]

Although pegaptanib (Macugen®; Eyetech Pharmaceuticals Inc, FL, USA and Pfizer Inc, New York, NY, USA) was the first VEGF inhibitor approved by the US Food and Drug Administration (FDA). Important advances in the on-label treatment of CNV in AMD have been achieved with the introduction of ranibizumab (Lucentis; Genentech USA, Inc, San Francisco, CA, USA) in 2006. The off-label use of bevacizumab (Avastin; Genentech USA, Inc) has also shown efficacy for treating wet AMD and other exudative retinal diseases and despite the lack of clinical trials to support its safety or efficacy, anecdotal evidence led to its widespread popularity prior to the approval of ranibizumab.

Aflibercept (EYLEA®; Regeneron Pharmaceutical Inc, Tarrytown, NY, USA and Bayer, Basel, Switzerland), also named VEGF Trap-eye, is the most recent member of the anti-VEGF family. This drug has been recently developed to afford a more potent and prolonged anti-VEGF effect and was approved by the FDA in November 2011.[6] This article reviews the efficacy and summarizes the pharmacological properties of VEGF Trap-eye and describes the possible advantages of its use over the currently used "older" anti-VEGF drugs.

Overview of VEGF and its pathological effects in neovascular AMD

VEGF-A (usually simply referred to as VEGF) is a growth factor encoded by a gene family that also includes placental growth factor (PlGF), VEGF-B, VEGF-C, VEGF-D, and the orf virus encoded VEGF-E.[7] Differences in exon splicing result in the generation of four main VEGF isoforms: $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$, which have 121, 165, 189, and 206 amino acids after cleavage of the signal sequence, respectively.[8]

VEGF stimulates the growth of vascular endothelial cells derived from arteries, veins, and the lymphatic system.[9] It also induces the formation of thin-walled endothelium-lined structures (ie, angiogenesis) in a variety of in vivo models,[10] and induces rapid elevations in microvascular permeability.[11] VEGF acts also as a survival factor for endothelial cells, both in vitro and in vivo.[12,13] Although endothelial cells represent the primary target of VEGF, several studies have demonstrated that VEGF has mitogenic effects on nonendothelial cell types[14] and promotion effects on monocyte migration.[15] VEGF protects neurons from insults such as hypoxia and glutamate toxicity[16] and it stimulates neurogenesis in vitro and in vivo.[17]

VEGF contributes mainly at the initiation stage of CNV by promoting both angiogenesis and vasculogenesis. It acts as an endothelial cell specific mitogen as part of the angiogenesis pathway, and also as a chemoattractant for endothelial cell precursors, inducing their mobilization and differentiation in the vasculogenesis pathway.[16] In addition to these activities, VEGF affects vascular permeability by inducing formation of pores in vascular endothelial cells[17,18] and by disrupting the intercellular junction between these cells.[19] In turn, this leads to extravasation of fluid, proteins, and circulating cells which disrupts the retinal anatomy and separates the retina from underlying structures, potentially causing severe vision loss.

Although other growth factors can induce the development of blood vessels (ie, transforming growth factor-β, interleukins, insulin-like growth factor-1, and epidermal growth factor), only VEGF appears to be both sufficient and essential for physiologic and pathologic angiogenesis. For this reason, the biochemical pathways involving VEGF are the most studied targets for new potential drugs against neovascular pathologies. Anti-VEGF therapy can arrest choroidal angiogenesis and reduce vascular permeability, which is frequently the main cause of visual acuity deterioration. Pegaptanib and ranibizumab have been approved by the FDA for the treatment of wet AMD, and the off-label use of a third agent, bevacizumab, has shown efficacy for treating wet AMD and other exudative retinal diseases. Pegaptanib was the first anti-VEGF drug FDA approved in December 2004.[20–22] However, because it was proven to be less efficacious than other anti-VEGF drugs, possibly owing to its selective binding of $VEGF_{165}$, it is no longer widely used in most countries. Ranibizumab and bevacizumab, which are nonselective anti-VEGF drugs, are currently the most extensively used drugs worldwide for wet AMD as well as for many other ocular diseases in which VEGF is overexpressed.[23]

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The development of new agents for wet AMD has focused on both improving efficacy and extending the duration of action in comparison with the commonly used anti-VEGF drugs ranibizumab and bevacizumab, which are considered the standard drugs. Ranibizumab is a monoclonal humanized antibody fragment and bevacizumab is a whole monoclonal antibody, and both show a high binding affinity for all isoforms of VEGF. These agents appear to have similar efficacy profiles and mechanisms of action, ie, they block the extracellular availability of VEGF which can arrest choroidal angiogenesis and reduce vascular permeability for a limited period of time.[24–27]

Bevacizumab has a lower binding affinity for VEGF than ranibizumab.[28] However, bevacizumab is approximately three times larger than ranibizumab (149 kDa versus 48 kDa), and its substantially higher molecular weight results in an intravitreal half-life that is 36% higher than that of ranibizumab. Accumulating clinical evidence has demonstrated that the effects of a single intravitreal dose of either bevacizumab or ranibizumab effectively reduces the effect of VEGF on CNV for 4–6 weeks in most eyes.[29,30]

Ranibizumab, which is the only widely used drug that is currently approved by the FDA for the treatment of neovascular AMD, is most extensively studied. Several ranibizumab Phase III clinical trials that have studied different treatment schedules, doses, and populations have obtained good results with monthly injections, ie, a mean number of 25 intravitreal injections over 2 years.[31,32]

Despite the off-label status of bevacizumab, however, it is preferred over ranibizumab by nearly 60% of physicians[33] because of its significantly lower price (ranibizumab, US $1,950 versus bevacizumab, US $50) and similar efficacy. The FDA originally approved bevacizumab in 2004 for the treatment of metastatic colorectal cancer.[34] To deliver an intravitreal injection, the physician or pharmacist makes numerous unit doses from a vial of bevacizumab, dramatically lowering the cost of the drug. Moreover, many reports and a 2-year multicenter, randomized clinical trial (the Comparisons of Age-Related Macular Degeneration Treatment Trial [CATT]) demonstrated its near equivalency to ranibizumab with monthly dosing (+7.8 letters versus +8.8 letters) and insignificant poorer outcomes with as-needed dosing (+5.0 versus +6.7 letters).[24,25] Moreover, while the systemic half-life of the unbound product of bevacizumab (20 days) was longer than that of ranibizumab (6 hours), severe systemic adverse events occurred at similar frequencies in patients receiving bevacizumab and ranibizumab in the CATT trial.[26,35,36]

The main problem with the current anti-VEGF therapy is that monthly intravitreal injections are required for maintaining vision. This necessitates an excessive time commitment from patients and institutions, and increases the physical and psychological discomfort and financial burdens for the patients. On the other hand, evidence from the SAILOR (Safety Assessment of Intravitreous Lucentis fOR AMD),[37] PIER (A Phase IIIb, Multicenter, Randomized, Double-Masked, Sham Injection-Controlled Study of the Efficacy and Safety of Ranibizumab in Subjects with Subfoveal Choroidal Neovascularization [CNV] with or without Classic CNV Secondary to Age-Related Macular Degeneration),[38,39] and EXCITE (Efficacy and Safety of Ranibizumab in Patients With Subfoveal Choroidal Neovascularization [CNV] Secondary to Age-Related Macular Degeneration)[40] studies indicates that the efficacy decreases if treatment frequency is reduced. After the loading dose of monthly injections for 3 months of ranibizumab, vision decreases or returns to baseline in most patients if the frequency is reduced to one injection every 2, 3, or 4 months.

Although monthly injections of anti-VEGF represent the best way to preserve vision, most retina surgeons use individualized treatment protocols with monthly assessments after the first three intravitreal injections of anti-VEGF, and further injections are given only if signs of disease activity persist as observed on optical coherence tomography (OCT). This strategy is also abbreviated as "PRN dosing" from the Latin phrase Pro Re Nata, which means "as circumstances arise." The PrONTO (Prospective Optical Coherence Tomography [OCT] Imaging of Patients With Neovascular AMD Treated With Intra-Ocular Ranibizumab) study used this strategy and obtained visual outcomes similar to those achieved with monthly injections while reducing the number of injections from 25 to 10 over 2 years.[41] However, even with this dosing regimen, patients are still required to make monthly visits to the office and undergo frequent and expensive testing because of the constant risk of CNV recurrence.

A treatment approach that aims to reduce the number of injections and the number of visits is the "treat and extend" method. It consists of 3 monthly injections and a follow-up examination after 6 weeks. If the follow-up examination shows evidence of exudation, the patient is treated and told to undergo a follow-up examination in 4 weeks, otherwise the patient is still treated but the follow-up period is extended to 8 weeks. A similar evaluation is performed at the next follow-up visit. However, there is not much evidence in favor of this treatment method. Thus, research on new compounds is focused on inhibiting the VEGF signaling pathway for a more prolonged period.[1]

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Aflibercept (EYLEA®; Regeneron Pharmaceutical Inc and Bayer), or VEGF Trap-eye, is a novel compound derived from the native VEGF receptor (VEGFR) that binds to all VEGF and VEGF-B isoforms as well as to PlGF.[42] VEGF Trap-eye promises to decrease the injection frequency in conjunction with the "treat and extend" or "PRN" strategies and appears to serve as an effective alternative drug for patients who are less responsive to the previously approved anti-VEGF drugs.

Structure and mechanism of action

The FDA approved VEGF Trap-eye (EYLEA®, Regeneron Pharmaceutical Inc, and Bayer) for the treatment of subfoveal CNV caused by wet AMD on November 18, 2011.[43] VEGF Trap-eye is an intraocular formulation of aflibercept, a product used in oncology (Zaltrap; Regeneron Pharmaceutical Inc), that has been specifically purified and buffered to minimize the risk of eye toxicity when injected intravitreally.[44] It is a fully human, recombinant fusion protein that has the property to "trap," that is to catch, hold, and block certain molecules. Aflibercept was constructed from portions of the human VEGFR fused to the FC portion of a human IgG1.[45]

Circulating VEGF initiates a biochemical cascade by activating three membrane spanning tyrosine kinase receptors: VEGFR-1, VEGFR-2, and VEGFR-3.[46,47] VEGFR-1 (fms-like tyrosine kinase-1, Flt-1) was the first VEGF receptor identified more than a decade ago.[48] VEGFR-1 releases tissue specific growth factors, recruits endothelial progenitors, and induces matrix metalloproteinases. It is thought to modulate VEGFR-2 signaling and to act as a dummy/decoy receptor by sequestering VEGF and preventing it from binding to VEGFR-2.[7] VEGFR-2 (kinase insert domain-containing receptor or KDR) is considered the major mediator of the mitogenic, angiogenic, permeability enhancing, and anti-apoptotic effects of VEGF.[7]

Both VEGFR-1 and VEGFR-2 have seven Ig-like binding sequences for VEGF (two of which are incorporated in VEGF Trap-eye) in the extracellular region, a single transmembrane region, and a consensus tyrosine kinase sequence that is interrupted by a kinase insert domain.[49–51] The third member of the same family of receptor tyrosine kinases is VEGFR-3.[52] This protein is not a receptor for VEGF, but binds VEGF-C and VEGF-D.[53] Because VEGFR-1 possesses a higher affinity for VEGF than VEGFR-2, drug developers have used its binding sequences for VEGF Trap-eye.

Structurally, aflibercept is a soluble decoy receptor of 115 kDa that is made by the second binding domain of VEGFR-1 and the third binding domain of VEGFR-2, which then are fused to the FC region of a human IgG1 (Figure 1).

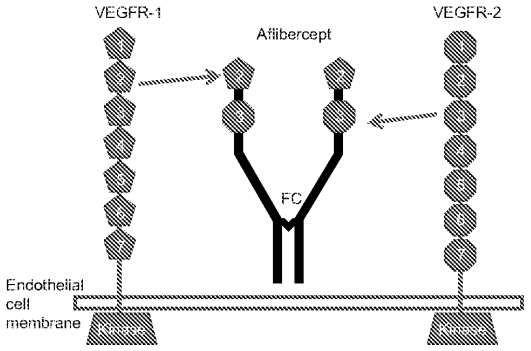


Figure 1 Diagram showing the structure of the vascular endothelial growth factor receptor-1 and -2 and the structure of aflibercept (VEGF Trap-eye).
Notes: Aflibercept (VEGF Trap-eye) is generated by a fusion that includes the second binding domain of vascular endothelial growth factor receptor (VEGFR)-1 and the third binding domain of VEGFR-2 attached to a FC fragment of a human IgG.
Abbreviation: FC, fragment crystallizable region.

The intermediate size of aflibercept (115 kDa compared to 48 kDa for ranibizumab and 148 kDa for bevacizumab) results in an estimated intravitreal half-life of 7.1 days and a duration of clinical action possibly as long as 2.5 months, which exceeds the 1-month intravitreal binding activity of ranibizumab.[54,55] The molecular configuration of aflibercept allows it to bind to all of the VEGF isoforms more tightly than their native receptors (the dissociation constant [K_d] of aflibercept for $VEGF_{165}$ = 0.49 pmol/L).[42] Thus, this compound effectively prevents VEGF from binding and activating its cognate receptors (the K_d of VEGFR-1 and VEGFR-2 for $VEGF_{165}$ are 9.33 and 88.8 pmol/L,

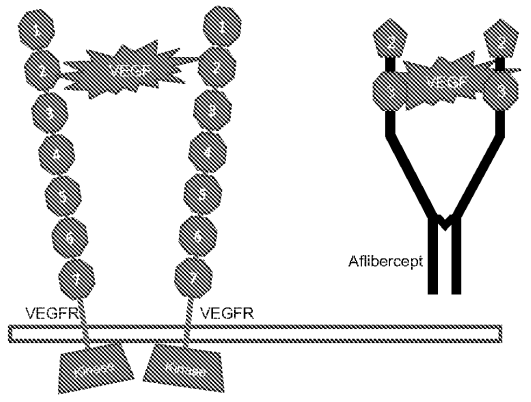


Figure 2 Vascular endothelial growth factor binds to two vascular endothelial growth factor receptors which induces the angiogenic response by activating the tyrosine kinase.
Notes: Vascular endothelial growth factor receptor (VEGFR)-2 is shown. Aflibercept (VEGF Trap-eye) binds all vascular endothelial growth factor (VEGF) isoforms more tightly than their native receptors, thus preventing binding of VEGF to its cognate receptors.

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respectively) (Figure 2).[56] Moreover, the binding affinity of aflibercept (K_d = 0.49 pmol/L) is almost 100 times higher than that of ranibizumab (K_d = 46 pmol/L) and bevacizumab (K_d = 58 pmol/L).[54,55] This was primarily attributed to the association rate constant for aflibercept binding to human $VEGF_{165}$, which is almost 80 times faster than the corresponding association rate constant values for ranibizumab and bevacizumab.

Because of these characteristics, the ability of aflibercept to block VEGF induced activation of VEGFR-1 and -2 in vitro is much stronger than that of ranibizumab and bevacizumab. Additionally, it blocks both PlGF-1 and PlGF-2 mediated activation of VEGFR-1, whereas ranibizumab and bevacizumab do not show such activity. A presumably important functional difference between aflibercept and the other anti-VEGF drugs currently in use is that it can bind and inhibit VEGF as well as PlGF-1 and -2 and VEGF-B, which have also been implicated in pathological vascular remodeling. Experimental evidence shows that targeting VEGF-B and PlGF inhibits CNV and suggests that PlGF synergizes with VEGF in promoting vascular pathology in wet AMD.[57]

Pharmacodynamics, pharmacokinetics, and metabolism

Aflibercept forms a stable, inert 1:1 complex with either VEGF, VEGF-B, or the PlGF ligand preventing the activation of their receptors, VEGFR-1 and -2.[56] The highest intravitreal dose used in pivotal trials for aflibercept is 2 mg, which is 100-fold lower than the dose allowed in oncology (4–6 mg/kg).[44,60] Following intravitreal injection of 2 mg of aflibercept, the drug can be detected in plasma as a free drug (a minor quantity) or in a complex bound with VEGF. The drug is rapidly cleared from circulation via pinocytotic proteolysis and glomerular filtration after forming a complex with VEGF via the same pathways that metabolize antibodies.

Following intravitreal injection of 2 mg of aflibercept, the mean maximal plasma concentration of unbound VEGF Trap-eye is attained in 1–3 days, and was estimated to be 200-fold lower than the concentration required for maximal systemic VEGF binding. The systemic half-life of unbound aflibercept is 1.5 days, which is inferior to that of bevacizumab (20 days) and closer to the systemic half-life of ranibizumab (6 hours).[59] Free aflibercept has never been detected in plasma at 2 weeks after intravitreal injection and cannot accumulate in plasma in the loading phase.[44] Thus, an intravitreal aflibercept dose of 2 mg would be predicted to cause negligible systemic activity and have a systemic safety profile similar to that of ranibizumab.

Therapeutic efficacy

The first surveys regarding the use of aflibercept in treatment of wet AMD emerged from a preclinical study conducted on animal models. This study, published in 2003, showed the first evidence that VEGF Trap-eye is capable of suppressing CNV and VEGF mediated breakdown of the blood–retinal barrier in transgenic mice with laser induced CNV, which was treated with subcutaneous or intravitreal administration.[58] The initial use of aflibercept for wet AMD consisted of intravenous injections with doses between 0.3 mg/kg and 3 mg/kg (the usual oncologic dose is 4 mg/kg) and administered every 2 weeks to 25 patients.[60] Macular thickness decreased by an average of 66% and vision improved in many patients. Patients receiving the higher dose (3 mg/kg) experienced more systemic hypertension and proteinuria than those treated with the lower dose (1 mg/kg). However, the promising effects obtained intravenously encouraged researchers to transition the trial to intravitreal injections.

The Phase I Clinical Evaluation of Anti-angiogenesis in the Retina Intravitreal Trial (CLEAR-IT 1)[60] investigation was a small trial (21 patients) designed to determine the maximum tolerated dose, the bioactivity, and the safety and tolerability of intravitreally administered aflibercept in patients with wet AMD. This study confirmed that aflibercept doses between 0.05 mg and 4 mg were well tolerated. At 6 weeks after a single injection, most patients experienced an improvement in visual acuity (mean visual gain, 4.4 letters) and showed a decrease in macular thickness (−105 μm). Almost 50% of the patients followed for 12 weeks did not show retinal leakage and maintained vision gain.[61,62] On the basis of the results of CLEAR-IT 1, the developers hoped to show that an intravitreal formulation of aflibercept could be administered less frequently than once a month.

In a Phase II dose and interval ranging trial, 159 patients with wet AMD (CLEAR-IT 2) were randomized into five treatment groups: the first two groups received 3 monthly aflibercept injections of 0.5 mg or 2 mg and the other three groups received only one aflibercept injection of 0.5 mg, 2 mg, or 4 mg.[64] Final global evaluations were performed at 12 weeks. Although visual improvement at week 8 was similar in patients receiving a single dose or two doses (5.7 letters), the average vision in all groups improved more in patients treated monthly (mean gain of ≥8 letters) at 12 weeks. After 12 weeks, the reduction in macular thickness experienced by the patients receiving three monthly injections

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exceeded that of patients treated only once.[63] For this reason, a second part of the CLEAR-IT 2 study was designed in which aflibercept treatment was provided as needed (PRN) from week 12 to 52 and monthly OCT and fluorescein angiography (FAG) examinations were performed, starting with a reinjection of all patients at week 12.[64] A decision to perform reinjection was made if any of the following conditions were observed: central retinal thickness increase of ≥100 μm, loss of at least five lines in the visual acuity chart approved by the Early Treatment Diabetic Retinopathy Study (ETDRS),[65] persistent fluid on OCT, new onset of classic neovascularization, persistent leakage on FAG, or the presence of a new hemorrhage on clinical examination. An average of two injections was required, with a mean time to the first injection of 129 days. After 1 year (week 52), the average improvement in vision was +5.3 letters. Patients initially treated with 2 mg every 4 weeks had the best visual improvement (mean gain of 9 letters).

The CLEAR-IT 2 study provided the first indication that aflibercept may be dosed as needed with excellent gains in vision.[66] Additionally, patients receiving a monthly "loading" dose for 3 months achieved superior visual results than those receiving single injections. Many patients required only two injections after the loading phase and at the last visit after 1 year. Thus, three different dosing regimens were identified for the Phase III studies:[67] 0.5 mg monthly, 2 mg monthly, or 2 mg every 2 months after the loading phase of three initial monthly doses.

In Phase III, two equivalent pivotal clinical trials of VEGF Trap-eye, VEGF Trap-eye Investigation of Efficacy and Safety in Wet AMD (VIEW) 1 and 2, were conducted to determine if VEGF Trap-eye was noninferior and clinically equivalent to ranibizumab, the drug considered to be the standard against which all subsequent drugs should be compared.[66,67] The VIEW 1 study enrolled 1,217 patients in the US and Canada, and the VIEW 2 study enrolled 1,240 patients in Europe, Asia, Japan, and Latin America. Each trial randomized patients among three treatment regimens: 0.5 mg of aflibercept given monthly, 2 mg given monthly, and 2 mg given every two months after 3 monthly loading doses for 3 months. Both studies evaluated the noninferiority efficacy in comparison with a fourth arm of the study in which patients received 0.5 mg of ranibizumab monthly. The first noninferiority endpoint was the percentage of patients who maintained their visual acuity (decrease in vision less than −15 letters); the second noninferiority endpoint was the percentage of patients who gained vision.

After the first year, both the VIEW 1 and 2 studies were continued for a second year (52–96 weeks) in which a modified PRN strategy was adopted. Patients were assessed monthly and were treated only if necessary (with the same drug and dose as in the first year), but the injection was repeated at least every three months in all cases. At week 52, the proportion of patients who maintained their vision (lost <15 ETDRS letters) was approximately 95% when using 2 mg of aflibercept (either monthly or every 2 months after the loading phase). The same results were obtained with 0.5 mg of ranibizumab given monthly. The gains in vision were comparable among the drugs administered monthly: a mean gain of +10.9 letters and +7.6 letters in the aflibercept group and a mean gain of +8.1 letters and +9.4 letters in those receiving ranibizumab, in VIEW 1 and VIEW 2,[67] respectively.

In VIEW 1, patients receiving 2 mg of aflibercept every 4 weeks gained more vision than those receiving ranibizumab (+10.9 letters versus +8.1 letters; $P = 0.0054$).[67] Improvements in macular thickness were not statistically different among any of the treatment groups. VIEW 2 patients receiving 2 mg of aflibercept every 8 weeks showed bimonthly fluctuations in macular thickness without corresponding fluctuations in visual acuity.[67] The safety of aflibercept was excellent and was comparable with that of ranibizumab in both the VIEW 1 and VIEW 2 studies. Severe extraocular adverse events such as stroke and myocardial infarction occurred with similar frequencies in patients receiving aflibercept (0.7% and 2.6%, respectively) and in patients receiving ranibizumab (1.6% and 2.6%, respectively) in both VIEW trials.

In VIEW 1, the mean vision gain from the baseline (best corrected visual acuity) BCVA at week 52 was greater in the 2 mg aflibercept every month group when compared with the ranibizumab group (mean gain of +10.9 versus +8.1 ETDRS letters).[67] Conversely, a statistically significant difference was not found in vision gain in comparison to ranibizumab (mean gain of +7.6 letters versus +9.4 letters) in VIEW 2.[67] The reason for this difference in vision results is unknown. However, it is likely that racial and ethnic differences existed between the two trials. Several reports have suggested that the incidence of polypoidal choroidal vasculopathy, which has been suggested to be a variant of neovascular AMD, is markedly high in African-American people, relatively high in the Asian population, and low in white people with AMD.[68,69] Polypoidal CNV does not respond well to anti-VEGF therapy alone and should be treated with a combination of photodynamic therapy and anti-VEGF therapy for better results. Thus, a limitation of the two trials was the inclusion of all CNV types by using FAG but not indocyanine green angiography.

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A comparative subanalysis of the data will be required to address this difference.

However, both VIEW studies showed that 2 mg injections of VEGF Trap-eye every two months delivered a comparable gain in visual acuity to monthly ranibizumab (+7.9 versus +8.1 letters in VIEW 1; +8.9 versus +9.4 letters in VIEW 2).[67] Additional efficacy was not demonstrated when VEGF Trap-eye was administered every 4 weeks compared with every 8 weeks, thus suggesting that patients would not require monthly examinations. In the two trials, approximately one third of patients receiving 2 mg of aflibercept every second month experienced a clinical improvement in visual acuity (ranging from +7 to +10 letters). Based on the 1-year efficacy (maintenance of vision) and safety results of the VIEW trials, the FDA approved a regimen of 2 mg of VEGF Trap-eye every 8 weeks for the treatment of wet AMD.[70] The recommended treatment regimen includes three loading injections at 4-week intervals, followed by injections every 8 weeks. During the second year (52–96 weeks), patients were assessed monthly and, if necessary, were treated via a modified PRN protocol with a new injection performed not less frequently than once every three months. Between weeks 52 and 96, patients initially receiving 2 mg of aflibercept every 8 weeks and those initially receiving ranibizumab every 4 weeks maintained previous gains in vision.

In an integrated analysis of the VIEW 1 and VIEW 2 studies,[70] the visual acuity gain from baseline in the aflibercept group that received 2 mg every 8 weeks was +7.6 letters at week 96 compared to +8.4 letters at week 52, with an average of 11.2 injections over 2 years and 4.2 injections during the second year. The visual acuity gain from baseline in the monthly ranibizumab group was +7.9 letters at week 96 compared to +8.7 letters at week 52, with an average of 16.5 injections over 2 years and 4.7 injections during the second year.[70]

Only 16% of the patients received six or more injections during the second year.[70] In comparison, patients receiving ranibizumab monthly during the first year and PRN the second year received an average of 16.5 injections: 12 during the first year and an average of 4.7 injections over the second year. Approximately 26.5% of the patients required six or more injections during the second year. During year 2 of the VIEW trials,[70] 48% of the patients receiving 2 mg of aflibercept and 40% of the patients receiving ranibizumab received the minimum number (three) of injections.

In both studies,[67] the ocular adverse events experienced across the four treatment groups were those commonly associated with intravitreal injections:[35,36] conjunctival hemorrhages, eye pain, and vitreous floaters. Systemic adverse events, such as falls, pneumonia, cancer, and cardiovascular disease were also balanced across the groups and were those commonly found in elderly AMD patients. No evidence of an increased risk of thromboembolic events such as stroke or myocardial infarction was found.[71]

VEGF Trap-eye: other clinical uses in retinal disease

The VEGF cytokine also plays an important role in the pathogenesis of vascular retinal diseases like diabetic retinopathy, central retinal vein occlusion (CRVO), and branch retinal vein occlusion. It causes an increase in retinal capillary permeability and leakage of fluid into the retina and macula, leading to significant loss of central vision.[72] VEGF expression, which is upregulated by hypoxia, was found to be elevated in the ocular fluids of patients with diabetic macular edema (DME) and CRVO.[73] Anti-VEGF compounds have been successfully used as the first line of treatment for diabetic retinopathy[74] and macular edema due to CRVO, and have replaced laser photocoagulation in some cases.[75]

Several anti-VEGF agents have been evaluated in numerous clinical trials from 2008 to the present day. Most notably, these include prospective clinical trials regarding intravitreal ranibizumab for the treatment of DME in RD (READ2 [Ranibizumab for Edema of the mAcula in Diabetes], RESOLVE [Safety and Efficacy of Ranibizumab in Diabetic Macular Edema With Center Involvement], RESTORE [A 12 Month Core Study to Assess the Efficacy and Safety of Ranibizumab (Intravitreal Injections) in Patients With Visual Impairment Due to Diabetic Macular Edema and a 24 Month Open-label Extension Study], RISE [A Study of Ranibizumab Injection in Subjects With Clinically Significant Macular Edema (ME) With Center Involvement Secondary to Diabetes Mellitus (RISE)], RIDE [A Study of Ranibizumab Injection in Subjects With Clinically Significant Macular Edema (ME) With Center Involvement Secondary to Diabetes Mellitus (RIDE)]),[76] which demonstrated the superiority of this anti-VEGF compound over both sham injection and focal grid laser.[77–82] Aflibercept was evaluated in a double-masked, prospective, randomized, multicenter Phase II trial, entitled DME And VEGF Trap-eye: INvestigation of Clinical Impact (DA VINCI),[83,84] in which 221 patients with clinically significant DME with central macular involvement were randomized and 219 patients were treated with a balanced distribution over five groups. These groups included monthly doses of 0.5 or 2 mg of VEGF Trap-eye, monthly doses of 2 mg of VEGF Trap-eye for 3 months and then

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every 8 weeks, monthly doses of 2 mg of VEGF Trap-eye for 3 months and then PRN, and macular laser therapy.[83,84] The mean improvements in BVCA at 52 weeks in the VEGF Trap-eye groups were +11.0, +13.1, +9.7, and +12.0 letters, respectively, versus −1.3 letters in the laser group. It is interesting to note these similar results with longer dosing intervals of treatment.

The DA VINCI study[83,84] showed that in addition to the benefits related to the reduction of central macular edema, aflibercept provides secondary benefits related to the nonprogression of retinopathy with the prevents the development of vascular neoproliferation.[84] Aflibercept has turned out to be a promising option in DME therapy because of its high binding affinity and extended duration of action. The latter quality is very important in view of the fact that diabetic retinopathy is a chronic disease and that a large percentage of affected patients are of working age.

Presently, no published randomized clinical trials have directly compared any of the anti-VEGF drugs for the treatment of diabetic retinopathy. However, two Phase III clinical studies, the VIVID (VEGF Trap-Eye In Vision Impairment Due to DME)[85] and the VISTA (Study of Intravitreal Administration of VEGF Trap-Eye in Patients With Diabetic Macular Edema)[75] studies, have been initiated and are evaluating the efficacy and safety of VEGF Trap-eye in comparison with laser treatment over a period of 1 and 2 years, respectively. Finally, a three arm study comparing ranibizumab versus bevacizumab versus aflibercept – the DRCR protocol T – is now in the enrollment phase.[75]

In September 2012, the FDA approved aflibercept injection for the treatment of macular edema following CRVO.[86] This approval was based on data from the Phase III COPERNICUS (Controlled Phase 3 Evaluation of Repeated intravitreal administration of VEGF Trap-Eye In Central retinal vein occlusion: Utility and Safety)[87,88] and GALILEO (General Assessment Limiting Infiltration of Exudates in central retinal vein Occlusion with EYLEA) studies.[89] In both studies, the results regarding the quality of vision and anatomical outcomes were superior in the aflibercept treated group than in the sham control group. The initial 6-month phase was similar among these studies, during which patients were randomized to receive either an intravitreal injection of 2 mg of aflibercept or a sham injection every month, but the second 6-month phase was different between the two studies. In the GALILEO study,[89] patients in the treatment group were treated on a PRN basis with aflibercept, while patients in the placebo group continued to receive treatment with sham injections; in the COPERNICUS study,[88] all patients were treated with aflibercept on a PRN basis.

In both the COPERNICUS and GALILEO studies, aflibercept injection resulted in an improvement in visual acuity of >15 letters in 56.1% and 60.2% of patients, respectively, at week 24 compared with those receiving sham injections (12.3% and 22.1%, respectively).[87,89] At week 52 in the COPERNICUS study,[88] the improvement in visual acuity was 55.3% in the aflibercept/aflibercept PRN patients compared with 30.1% in the sham/aflibercept PRN patients. In the GALILEO study, in which control patients did not receive any aflibercept injections, the improvement was 60.2% and 32.4%, respectively.[88,90] The results of these studies showed that it is possible to maintain an excellent visual outcome and to extend the range of administration while using the PRN strategy. These data indicate that aflibercept provides benefits to patients with CRVO and using this drug as needed may become a first line approach that will reduce the burden of monthly injections.

Conclusion

In conclusion, aflibercept, or VEGF Trap-eye, may be considered an attractive alternative to other anti-VEGF agents because it appears to offer visual outcomes similar to ranibizumab and bevacizumab with a longer duration of action. For the first time, an anti-VEGF drug can be given at 2-month intervals with results comparable to ranibizumab given every 4 weeks.[91]

Aflibercept was shown to be generally well tolerated in the VIEW I and II studies, and the ocular adverse events and adverse events were similar to those of ranibizumab. Patients receiving 2 mg of aflibercept every 8 weeks achieved visual acuity gains similar to those receiving ranibizumab with five fewer injections, on average, over 2 years. Patients who required the most intense therapy received, on average, 1.4 fewer injections in the group receiving 2 mg of aflibercept every 8 weeks when compared to the ranibizumab group in the second year.

Although the future direction of the development of therapeutic management techniques should be driven by improving results, reducing the burden and the cost of treatment should also be considered. In particular, the cost of AMD treatments with the approved anti-VEGF agents is much higher by any metric compared to any previous AMD and retinal treatment. Economic consideration is an important influencing factor in the selection of drugs for individual patients, and the comparable safety and reduced injection burden of aflibercept in comparison with ranibizumab enhances its cost effectiveness. For those clinicians using ranibizumab, the transition to

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aflibercept (which costs $100 less than ranibizumab) will be easy because the total cost of aflibercept treatment will be even lower than the presumed per vial cost after accounting for the fact that the cost will be lowered further by the greater time interval between injections. However, the transition to aflibercept from off-label bevacizumab (which costs $1,800 less than aflibercept) will be slower for cost conscious physicians. In this case, the relative merits of the more expensive, but less frequently dosed, aflibercept compared to the more frequently dosed, lower cost alternative of off-label bevacizumab must also be considered.

Moreover, aflibercept can be used in shifting patients treated with bevacizumab to aflibercept, as this monthly injection was the only regimen shown to be equivalent to ranibizumab in the comparison of AMD treatment trials.[25] Yet another strategy woven into combination therapy stems from the observation that most visual improvements with anti-VEGF agents occur in the first three months, raising the possibility of an initial (albeit high cost) loading treatment with a subsequent (lower cost) maintenance treatment. The addition of new drugs to these combination strategies may diminish both maintenance and loading therapies, achieving better results.

Furthermore, a major concern with chronic therapies is the reduction of the biological effect, which can limit long-term efficacy. This phenomenon has been called tachyphylaxis and has been described as a progressive decrease in the therapeutic response after repetitive administration of anti-VEGF drugs.[92] A retrospective review from the National Eye Institute found that between five and ten injections of bevacizumab were required before tachyphylaxis occurred.[93] Nonresponder patients, or patients who experience tachyphylaxis, will need alternative treatment strategies to break the cycle of monthly injections with the same stagnant results. A possible solution would be to combine drugs with different mechanisms of action or different pharmacokinetics, for example, switching the treatment to different VEGF blockers. Several reports have shown that administration of aflibercept to eyes that had persistent fluid despite prolonged bevacizumab or ranibizumab therapy resulted in rapid resolution of the subretinal fluid and the flattening of pigment epithelial detachments.[6] This indicates that aflibercept can be used with success in patients who show resistance to conventional anti-VEGF drugs and suggest that aflibercept works remarkably well as a "salvage" therapy.

In light of the above analysis based on the literature, the personal opinion of the authors on the therapy for maculopathy is that the best approach for wet AMD is an "attack on several fronts." In this sense, the first line drugs are anti-VEGF agents that can be used in combination with drugs that inhibit the actions of molecules involved in angiogenesis, including integrins, complements, and PlGF, and with compounds that are able to maintain and preserve the integrity of the retinal photoreceptors and of the choriocapillaris. However, because an effective combination therapy is still several years away, aflibercept promises to become the leading medication in the treatment of wet AMD in the coming years because of its ability to inhibit angiogenesis.

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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 randomised trial

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Summary

Background Docetaxel plus prednisone is standard first-line chemotherapy for men with metastatic castrate-resistant prostate cancer. Aflibercept is a recombinant human fusion protein that binds A and B isoforms of VEGF and placental growth factor, thereby inhibiting angiogenesis. We assessed whether the addition of aflibercept to docetaxel and prednisone would improve overall survival in men with metastatic castrate-resistant prostate cancer compared with the addition of placebo to docetaxel and prednisone.

Methods VENICE was a phase 3, multicentre, randomised double-blind placebo-controlled parallel group study done in 31 countries (187 sites). Men with metastatic castrate-resistant prostate cancer, adequate organ function, and no prior chemotherapy were treated with docetaxel (75 mg/m² intravenously every 3 weeks) and oral prednisone (5 mg twice daily) and randomly allocated (1:1) to receive aflibercept (6 mg/kg) or placebo, intravenously, every 3 weeks. Treatment allocation was done centrally via an interactive voice response system, using a computer-generated sequence with a permuted-block size of four and stratified according Eastern Co-operative Group performance status (0–1 *vs* 2). Patients, investigators, and other individuals responsible for study conduct and data analysis were masked to treatment assignment. Aflibercept or placebo vials were supplied in identical boxes. The primary endpoint was overall survival using intention-to-treat analysis. This is the primary analysis of the completed trial. The study is registered with ClinicalTrials.gov, number NCT00519285

Findings Between Aug 17, 2007, and Feb 11, 2010, 1224 men were randomly allocated to treatment: 612 to each group. At final analysis, median follow-up was 35 months (IQR 29–41) and 873 men had died. Median overall survival was 22·1 months (95·6% CI 20·3–24·1) in the aflibercept group and 21·2 months (19·6–23·8) in the placebo group (stratified hazard ratio 0·94, 95·6% CI 0·82–1·08; p=0·38). We recorded a higher incidence of grade 3–4 gastrointestinal disorders (182 [30%] *vs* 48 [8·0%]), haemorrhagic events (32 [5·2%] *vs* ten [1·7%]), hypertension (81 [13%] *vs* 20 [3·3%]), fatigue (97 [16%] *vs* 46 [7·7%]), infections (123 [20%] *vs* 60 [10%]) and treatment-related fatal adverse events (21 [3·4%] *vs* nine [1·5%]) in the aflibercept group than in the placebo group.

Interpretation Aflibercept in combination with docetaxel and prednisone given as first-line chemotherapy for men with metastatic castrate-resistant prostate cancer resulted in no improvement in overall survival and added toxicity compared with placebo. Docetaxel plus prednisone remains the standard treatment for such men who need first-line chemotherapy.

Funding Sanofi and Regeneron Pharmaceuticals Inc.

Introduction

Most men with metastatic prostate cancer respond to androgen-deprivation therapy with orchiectomy or a gonadotropin-releasing hormone agonist, but the disease progresses to a castration-resistant state. Secondary responses to hormonal agents such as androgen-receptor inhibitors can occur after progression on primary androgen-deprivation therapy, and secondary responses to androgen-deprivation therapy might become more frequent as new inhibitors of androgen synthesis (eg, abiraterone acetate[1]) or androgen-receptor signalling (eg, enzalutamide[2]) are used.

Men with metastatic castration-resistant prostate cancer can benefit from chemotherapy. Mitoxantrone was the first chemotherapy approved for such men, based on findings that showed improved pain control in trials that compared treatment with mitoxantrone and corticosteroid with corticosteroid alone.[3,4] Since 2004, standard first-line chemotherapy has been docetaxel (75 mg/m² every 3 weeks) and oral prednisone or prednisolone (5 mg twice daily), after demonstration of improved survival compared with mitoxantrone and prednisone.[5,6] Findings from this trial also showed better pain control, prostate-specific antigen (PSA), and

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health-related quality-of-life response in men randomised to receive docetaxel and prednisone,[5–7] and were lent support by findings from the SWOG-9916 trial, which also compared docetaxel-based chemotherapy with mitoxantrone and prednisone.[8]

Many phase 3 trials[9–15] have assessed the addition of targeted agents to docetaxel plus prednisone in attempts to improve overall survival in men with metastatic castrate-resistant prostate cancer. Agents assessed include DN101 (high-dose calcitriol), G-VAX vaccine (Cell Genesys, South San Francisco, CA, USA), atrasentan, zibotentan (inhibitors of endothelin-1 receptor-A), lenalidomide, bevacizumab (an inhibitor of VEGF), and dasatinib (an inhibitor of the Src protein and other tyrosine kinases), but none of these agents improved survival, and all of them added toxicity. Another trial assessing docetaxel and prednisone with custirsen (OGX-011; an inhibitor of clusterin synthesis) is ongoing (NCT01188187).

Aflibercept (also known as VEGF-Trap and ziv-aflibercept; Sanofi, Paris France; and Regeneron, Tarrytown, NY, USA) is a recombinant fusion protein consisting of extracellular domains of the human VEGF receptor (VEGFR) fused to the Fc portion of human immunoglobulin G1.[16,17] It contains sequences encoding the immunoglobulin domain 2 from VEGFR1 fused to the immunoglobulin domain 3 from VEGFR2, which in turn is fused to the hinge region of the human immunoglobulin G1 Fc domain. Aflibercept has high binding affinity to the isoform VEGF-A, and also binds VEGF-B and platelet-derived growth factors PlGF1 and PlGF2, thereby inhibiting angiogenesis.[16–18] Aflibercept has been assessed alone and with chemotherapy, including docetaxel, in preclinical models and showed activity against the DU 145 prostatic carcinoma in immune-deprived mice.[18] Aflibercept has been assessed in phase 1 and 2 clinical trials with docetaxel,[19,20] although no phase 2 trial of this combination has been done for men with metastatic castrate-resistant prostate cancer. We examined the combination of aflibercept with docetaxel and prednisone in the first-line treatment of such men. We aimed to show or exclude an improvement in overall survival compared with placebo with docetaxel and prednisone.

Methods

Trial design and participants

VENICE was a phase 3, multicentre randomised double-blind placebo-controlled parallel group study done in 31 countries (187 sites). Eligible participants had histologically or cytologically confirmed prostate cancer and evidence of metastatic disease that had progressed on hormonal therapy or after surgical castration. Criteria of progression before study entry were as defined by the Prostate Cancer Clinical Trials Working Group 2 (PCWG2):[21] the requirements were an increase in measurable disease, new lesions (more than two lesions if on bone scan), or two successive rises in serum PSA concentrations and an absolute value of 2 ng/mL or greater. Patients' serum testosterone needed to be 0·50 ng/mL or lower, and treatment with an agonist of luteinising-hormone-releasing hormone was continued in participants who were receiving it. If patients had initial complete androgen blockade, or had reduction in PSA for 3 months or more after addition of an anti-androgen, previous anti-androgen therapy was stopped 4–6 weeks before randomisation, depending on the agent used. Participants were required to have an Eastern Co-operative Group (ECOG) performance status of 0–2. Adequate organ and bone marrow function was determined by values of serum creatinine, aspartate, and alanine aminotransferases less than 1·5×upper limit of normal, bilirubin within the normal range, haemoglobin concentrations of 100 g/L or greater, an absolute neutrophil count of $1·5\times10^9$/L or greater, and a platelet count of 100×10^9/L or greater.

Men were ineligible if they had received previous cytotoxic chemotherapy for prostate cancer, except estramustine or adjuvant or neoadjuvant treatment completed 3 years or more before enrolment. They could not have received inhibitors of VEGF or its receptors. Previous treatment with radiotherapy, surgery, or estramustine must have been completed 28 days or more before randomisation, but patients receiving bisphosphonates were eligible. Participants were ineligible if they had received previous isotope therapy (eg, strontium-89 or samarium-153), whole-pelvic irradiation, or previous radiotherapy to more than 30% of the bone marrow. Patients who had no history of brain metastases, uncontrolled spinal-cord compression, or carcinomatous meningitis, and no active prior malignancy (except basal or squamous-cell skin cancer) within the previous 5 years were eligible. Other exclusion criteria included myocardial infarction, severe or unstable angina pectoris, coronary or peripheral artery bypass graft, congestive heart failure, cerebrovascular accident, or transient ischaemic attack within the previous 6 months; treatment-resistant peptic ulcer disease, erosive oesophagitis or gastritis, infectious or inflammatory bowel disease, diverticulitis, pulmonary embolism, or uncontrolled hypertension within 3 months or deep-vein thrombosis within 4 weeks of randomisation. Patients were also ineligible if they had active bleeding or grade 3 neuropathy, but those receiving warfarin with good control of anticoagulation were eligible. Participants were excluded if they had AIDS or known HIV disease needing antiretroviral therapy or any severe acute or chronic medical disorder. Men with reproductive potential, or their partners, were required to use contraception.

The institutional review boards in all participating centres approved the study, and men were required to sign the institutional review board-approved informed consent form before enrolment. Trial data were

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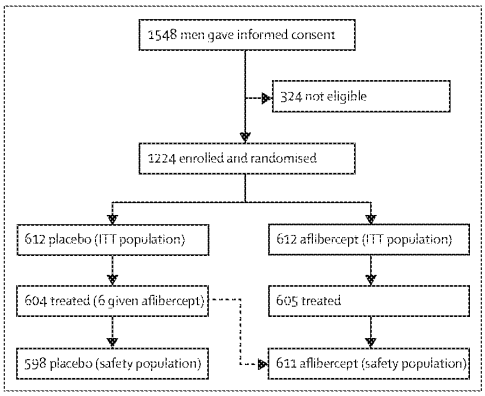


Figure 1: Trial profile
ITT=intention to treat.

monitored by an independent data monitoring committee, which met at 6-month intervals and provided recommendations to the study chair and trial committee.

Randomisation and masking

Generation of the patient randomisation list and management of treatment assignment was assigned to a third-party contractor: an interactive voice response system provider (S-CLINICA, Brussels, Belgium). Treatment was assigned centrally via the interactive voice response system, with patients randomly allocated in a one-to-one ratio to either the control or the experimental group, using a permuted-block size of four and stratified according ECOG performance status (0–1 *vs* 2). There was strict adherence to the randomisation list and no corrections allowed during the course of the study. The treatment code was released for study analysis only after the database was locked on Feb 7, 2012. Aflibercept or placebo vials were supplied in identical boxes corresponding to patient kits: each kit was labelled with a unique kit number. Patients, investigators, and all other people responsible for study conduct and data analyses were masked to treatment assignment. During the course of the study, an external statistician (independent from the sponsor) did unmasked safety and efficacy analyses (interim analyses) for the purpose of the independent data monitoring committee reviews. Access to these data and analyses was restricted to the committee members. A site was able to break the code for safety reasons only in exceptional circumstances (when knowledge of the investigational product was essential for treating the patient) by calling the interactive voice response system. If treatment allocation was revealed, the treating physician was to document the date and reason for code breaking in the case report form, and the patient discontinued the investigational product.

Procedures

Docetaxel (75 mg/m²) and aflibercept (6 mg/kg) or matched placebo, were administered intravenously every 3 weeks. Prednisone or prednisolone (5 mg) were administered orally twice daily, from day 1 continuously. Dexamethasone (or another corticosteroid) was given before and after docetaxel, and standard anti-emetics were provided. Use of granulocyte-colony stimulating factor was at the discretion of the treating clinician. Aflibercept was administered in 5 mmol/L phosphate, 5 mmol/L citrate, 100 mmol/L sodium chloride, 20% (weight to volume) sucrose, and 0·1% (weight to volume) polysorbate 20, pH 6·0, supplied in sealed, sterile, single-use 5 mL vials; contents of the vial were diluted before infusion. Placebo consisted of sterile aqueous buffered vehicle pH 6·0, which was otherwise identical to aflibercept. Aflibercept or matching placebo were given over 1 h on day 1 every 3 weeks, followed immediately by docetaxel over 1 h. For patients with a body surface area greater than 2·2 m², the dose of docetaxel was adjusted to a maximum body surface area of 2·2 m².

Dose adjustments or cycle delays were planned in case of toxicity. Aflibercept or placebo could be reduced to 3 mg/kg in the case of grade 3 hypertension not controlled by added medication after 2 weeks; it was discontinued if there was on going grade 3 hypertension or grade 3–4 thromboembolic events, haemorrhage, or gastrointestinal perforation. Docetaxel could be reduced to 60 mg/m² and further to 45 mg/m² in the event of febrile neutropenia, or grade 3–4 thrombocytopenia, stomatitis, or diarrhoea; one dose reduction was allowed for grade 2 peripheral neuropathy, cutaneous reactions, or increased liver enzymes, but treatment with the drug was stopped if these persisted. Once a dose had been decreased, a patient's dose could not be increased back to the previous level. No more than one treatment omission, and no more than 2 weeks of delay in treatment, were allowed: drugs were discontinued in patients needing greater dose reductions or delays due to toxicity. No decision to discontinue treatment was to be made for increase in PSA or pain alone within the first 12 weeks. Patients were to be treated for at least 12 weeks in the absence of clinical evidence of disease progression until progressive disease, unacceptable toxicity, or patient refusal of further study treatment. All patients were followed-up until death or the study cutoff date, whichever came first.

The primary outcome was overall survival. Key secondary endpoints were PSA response, time to first skeletal-related event, and progression-free survival. PSA response was defined (for patients with baseline PSA ≥10 ng/mL) as a 50% or greater decrease of serum PSA concentrations from baseline, confirmed at least 3 weeks later; increases in PSA during the first 12 weeks were ignored in the assessment of PSA response, as per recommendations of PCWG2.[21] Skeletal-related events included pathological fractures, spinal-cord compression,

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need for bone irradiation (including radioisotopes or bone surgery), and change of treatment (eg, introduction or change in route of administration of bisphosphonates to treat bone pain). Progression-free survival was defined as a composite endpoint including tumour progression, PSA progression, occurrence of a skeletal-related event, pain progression, and radiotherapy for cancer-related symptoms or death, whichever came first. Other secondary endpoints included tumour response (assessed by Response Evaluation Criteria In Solid Tumors criteria[22]), PSA progression-free survival (assessed by PCWG2 criteria[21]), and pain response and pain progression-free survival (assessed by the Present Pain Intensity Scale from the McGill-Melzack questionnaire and an analgesic consumption diary[5]). Data for health-related quality of life were obtained using the Functional Assessment of Cancer Therapy-Prostate[23] questionnaire and a Trial Outcome Index and will be reported separately.

Safety was assessed on the basis of treatment received by the frequency, severity, seriousness, and relation to treatment of treatment-emergent adverse events, which were assessed by clinical examination, including body weight, ECOG performance status and blood pressure, laboratory data (complete blood count, biochemistry, urinalysis, and other tests as indicated clinically), and concomitant drugs. We used the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0) to grade severity of adverse events.

Statistical analysis

The primary analysis of overall survival was comparison between the two treatment groups using a log-rank test stratified at randomisation by ECOG performance status. The study was designed to detect a hazard ratio (HR) of 0·80 with 90% power, which required 873 deaths; the planned sample size was 1200. Estimates of the HR and its (1–α)% CI (α being the two-sided nominal significance level of 0·044 at final analysis) were calculated with a Cox proportional hazard model stratified as described above; differences in overall survival were assessed by the stratified Kaplan-Meier method.

Exploratory analyses were done with Cox proportional hazard modelling to assess consistency of the treatment effect across subgroups and to examine the effect of covariates on overall survival. Covariates included age, ethnic origin, ECOG performance status, previous hypertension, on-going zoledronic acid treatment at study entry, time from initiation of hormonal therapy to progressive disease, and geographical region.

Two interim analyses of overall survival were planned for futility when 437 (50%) deaths had occurred and for early assessment of efficacy when 655 (75%) deaths had occurred. Type I and type II errors were protected by a group sequential approach with an O'Brien Fleming α-spending function and a non-binding gamma (–5) β-spending function.[24] The overall two-sided α nominal significance level for overall survival was 0·05.

	Aflibercept (N=612)	Placebo (N=612)
Age (years)	68 (43–88)	68 (40–87)
<65	195 (32%)	225 (37%)
65–74	283 (46%)	259 (42%)
≥75	134 (22%)	128 (21%)
White	560 (92%)	552 (90%)
Eastern Co-operative Group performance status		
0	283 (46%)	285 (47%)
1	303 (50%)	299 (49%)
2	26 (4·2%)	28 (4·6%)
Region		
Western Europe	227 (37%)	219 (36%)
Eastern Europe	132 (22%)	131 (21%)
North America	95 (16%)	81 (13%)
South America	71 (12%)	88 (14%)
Other	87 (14%)	93 (15%)
Previous prostatectomy	211 (35%)	214 (35%)
Previous radiotherapy		
Prostate	193 (32%)	202 (33%)
Bone	131 (21%)	134 (22%)
Stage at diagnosis		
Stage I–II	86 (14%)	82 (13%)
Stage III	81 (13%)	72 (12%)
Stage IV	321 (52%)	322 (53%)
Unknown	124 (20%)	136 (22%)
Gleason score		
2–4	22 (3·6%)	27 (4·4%)
5–7	263 (43%)	281 (46%)
8–10	295 (48%)	278 (45%)
Unknown	32 (5·2%)	26 (4·2%)
Years from diagnosis to randomisation	4·1 (0·4–18·6)	3·6 (0·3–21·5)
Previous hormonal therapy		
Number of regimens		
1	59 (10%)	73 (12%)
2	184 (30%)	167 (27%)
≥3	361 (59%)	361 (59%)
Unknown	8 (1·3%)	11 (1·8%)
Surgical castration	131 (21%)	147 (24%)
Gonadotropin-releasing hormone	526 (86%)	518 (85%)
Anti-androgen	567 (93%)	564 (92%)
Ketoconazole	37 (6·0%)	40 (6·5%)
Estramustine	32 (5·2%)	40 (6·5%)
On-going zoledronic acid	157 (26%)	165 (27%)
Criteria for progression		
Tumour progression	380 (62%)	372 (61%)
Increasing prostate-specific antigen concentration only	232 (38%)	239 (39%)
Sites of disease		
Bone	544 (89%)	541 (88%)
Lymph nodes	334 (55%)	330 (54%)
Visceral involvement	171 (28%)	176 (29%)
Prostate-specific antigen (ng/ml)	82·6 (0–6138)	92·9 (0–3821)

Data are median (range) or n (%)

Table 1: Baseline characteristics

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	Aflibercept (N=611)	Placebo (N=598)
Number of cycles administered	8 (1–50)	9 (1–36)
Number of cycles per patient		
1–3	137 (22%)	59 (10%)
4–6	131 (21%)	129 (22%)
7–9	131 (21%)	116 (19%)
10	62 (10%)	74 (12%)
11–15	98 (16%)	130 (22%)
>16	52 (8·5%)	90 (15%)
Median duration of treatment in weeks	24·0 (3–150)	28·9 (3–108)
Median relative dose intensity of aflibercept or placebo	0·97 (0·1–1·1)	0·99 (0·1–1·1)
Median relative dose intensity of docetaxel	0·93 (0·2–1·1)	0·97 (0·2–1·1)
Patients with at least one cycle delayed	302 (49%)	214 (36%)
Patients with at least one dose modification of aflibercept or placebo	65 (11%)	14 (2·3%)
Patients with at least one dose modification of docetaxel	189 (31%)	97 (16%)
Patients receiving granulocyte-colony stimulating factor	150 (25%)	89 (15%)
Reason for treatment discontinuation		
Adverse event	266 (44%)	127 (21%)
Disease progression	186 (30%)	334 (56%)
Investigator decision	47 (7·7%)	75 (13%)
Other reason	112 (18%)	62 (10%)

Data are median (range) or n (%).

Table 2: Treatment received (safety population)

We used a closed-test hierarchical procedure to control the type I error rate when analysing key secondary endpoints; we did formal statistical testing only if the difference in prior endpoint was statistically significant. The procedure was done in the following order: overall survival (primary endpoint), PSA response, time to skeletal-related event, then progression-free survival. We used stratified Kaplan-Meier methods to assess differences in time-to-event endpoints and stratified Cochran-Mantel-Haenszel to test for comparison of response rates. We used SAS (version 9.2) for all statistical analyses.

See Online for appendix

This study is registered with ClinicalTrials.gov, number NCT00519285

Role of the funding source

The study was designed through collaboration between employees of the sponsor (including EM) and the study chair (IFT), and cochairs (MR, BT, and DP). During the study, the primary data were collected and managed by the sponsor (including EM and LH); at study conclusion, data were analysed by statisticians employed by the sponsor (including LH), and the detailed clinical study report was primarily drafted by the sponsor (including EM) and made available to the study chair (IFT). At the conclusion of the trial, authors employed by the sponsor (EM and LH) had full access to the raw data. The article was drafted by the study chair and corresponding author (IFT), and was amended after review by all authors. The corresponding author had final responsibility to submit for publication.

Results

Between Aug 17, 2007, and Feb 11, 2010, 1224 patients with metastatic castrate-resistant prostate cancer were recruited from 187 centres in 31 countries: 612 patients were randomly allocated to each group (figure 1). Seven patients (1%) randomised to aflibercept and eight patients (1%) randomised to placebo were not treated: six patients in the placebo group received at least one dose of aflibercept in error (figure 1). The independent data monitoring committee recommended that the study continued without modification. The trial was analysed at median follow-up of 35 months (IQR 29–41) when 873 participants had died, as per the statistical plan.

Baseline demographic and clinical characteristics were much the same between the two groups (table 1). Most men had ECOG performance status 0–1 and most had received two or more lines of hormonal therapy. Most men had bone metastases, about half had lymph node metastases, and about a third had visceral disease (table 1).

More patients in the aflibercept group than in the placebo group stopped treatment because of an adverse event, resulting in a larger number of patients in the aflibercept than in the placebo group receiving three or fewer treatment cycles (table 2). Patients in the aflibercept group also had more delays and dose adjustments for both aflibercept and docetaxel, although the median dose intensity for aflibercept with placebo and docetaxel, calculated as a percentage of the ideal dose between first and last treatments, exceeded 93%. Further anticancer treatments administered after discontinuation of study treatment were much the same between treatment groups (appendix).

We recorded no between-group difference in median overall survival (HR for overall survival 0·94 [95·6% CI 0·82–1·08]; table 3 and figure 2). Exploratory analysis also showed survival to be similar for subgroups defined by ECOG performance status, age, geographical region, and other patient characteristics (data not shown).

More patients in the aflibercept group had a tumour response than did those in the placebo group, but we detected no between-group difference in any other of the secondary outcomes (tables 3 and 4), including time to first skeletal-related event and progression-free survival (table 3).

We recorded greater toxicity in the aflibercept group: grade 3–4 toxicities that were most common included gastrointestinal disorders such as diarrhoea, nausea and vomiting, stomatitis and ulceration, perforation, and fistula (table 5). Haemorrhage (mostly epistaxis), hypertension, fatigue, infection, neutropenia and its complications, dysphonia, and proteinuria were also more common in the aflibercept group than in the placebo group. The incidence of neuropathy and thromboembolic events was much the same between the

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	Aflibercept		Placebo		p value
	Number assessable	Median (95% CI)	Number assessable	Median (95% CI)	
Median overall survival (months)	612 patients (428 deaths)	22·1 (95·6% CI 20·3–24·1)	612 patients (445 deaths)	21·2 (95·6% CI 19·6–23·8)	0·38
Time to first skeletal-related event (months)	612 patients (497 events)	15·3 (14·1–16·7)	612 patients (516 events)	15·0 (13·7–16·4)	0·31
PFS (months)	612 patients (592 events)	6·9 (6·2–7·4)	612 patients (592 events)	6·2 (5·6–6·9)	0·31
PSA-PFS (months)	608 patients (567 events)	8·3 (7·8–8·8)	606 patients (571 events)	8·1 (7·6–8·6)	0·42
Pain-PFS (months)	287 patients (244 events)	9·2 (8·2–10·4)	301 patients (263 events)	9·7 (8·5–11·5)	0·87

Unless otherwise indicated figures in parentheses represent 95% confidence intervals. Except for the primary endpoint of overall survival, p values are descriptive and exploratory; they are not corrected for multiplicity. PFS=progression-free survival. PSA=prostate-specific antigen.

Table 3: Time to event efficacy outcomes, by treatment group

two groups. 34 (5·6%) patients in the aflibercept group and 20 (3·3%) died in the absence of disease progression, with 21 (3·4%) patients in the aflibercept group and nine (1·5%) in the placebo group judged to be related to treatment. The most common cause of fatal events in the absence of disease progression (and of the difference between the group) was infection (15 [2·5%] with aflibercept and four [0·7%] with placebo); other causes of fatal adverse events, including cardiac events, respiratory problems, and gastrointestinal events (including haemorrhage), were evenly distributed between the two groups. The median age of men with a fatal adverse event was 74 years (IQR 70–77) (compared with 68 years [62–74] for all participants).

Discussion

Findings from this large, international phase 3 trial showed no improvement in overall survival compared with placebo when aflibercept was added to docetaxel and prednisone to treat men with metastatic castrate-resistant prostate cancer. The median survival in both groups (21–22 months) was longer than anticipated for docetaxel and prednisone at the time the trial was designed (19 months, based on the results of the TAX327 trial[3]). This better-than-expected survival of men with metastatic castrate-resistant prostate cancer given chemotherapy probably reflects that men included in recent clinical trials are offered chemotherapy earlier in the course of their disease; it was probably not due to availability of more effective treatments after progression of disease on docetaxel, because few patients received new agents such as abiraterone, enzalutamide, or cabazitaxel that have been shown to improve survival (appendix).[1,2,25]

A limitation of our trial, and indeed of most clinical trials, is that entry criteria excluded patients with various types of comorbidity, which are common in elderly men with prostate cancer. Also, targeted agents generally add toxicity, and such toxicity is often underestimated from data in the original clinical trials that led to licensing of these agents.[26] Entry criteria for this trial were broadly similar to those for the TAX327 trial, which established docetaxel plus prednisone as standard first-line chemotherapy for men with metastatic castrate-resistant prostate cancer (panel). In a comparison of men with such cancer receiving first-line docetaxel plus prednisone within and outside of clinical trials at the Princess

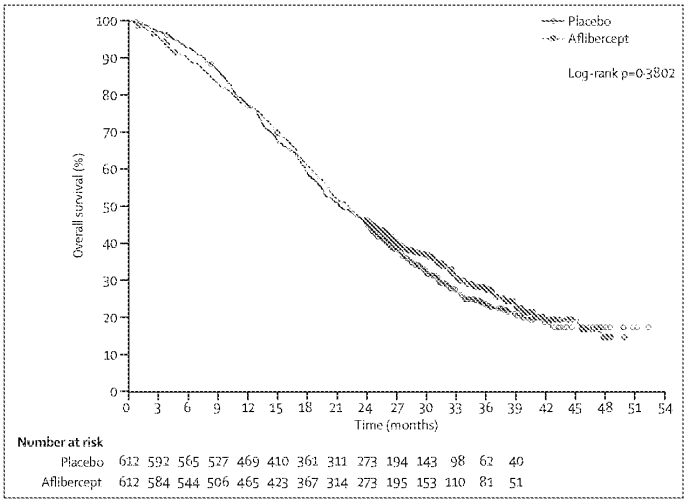


Figure 2: Kaplan-Meier curves for overall survival

	Aflibercept		Placebo		p value
	Number of events/ number assessable	% (95% CI)	Number of events/ number assessable	% (95% CI)	
PSA response	384/560	68·6 (64·7–72·4)	355/559	63·5 (59·5–67·5)	0·075
Tumour response	124/323	38·4 (33·4–44·0)	90/320	28·1 (23·2–33·1)	0·0043
Pain response	24/67	35·8 (24·3–47·3)	31/67	46·3 (34·3–58·2)	0·20

p values are descriptive and exploratory; they are not corrected for multiplicity. PSA=prostate-specific antigen.

Table 4: Other secondary outcomes

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	Aflibercept (N=611)		Placebo (N=598)	
	All grades	Grades 3–4	All grades	Grades 3–4
Any	607 (99%)	470 (77%)	585 (98%)	290 (49%)
Gastrointestinal disorders	513 (84%)	182 (30%)	409 (68%)	48 (8%)
Diarrhoea	276 (45%)	35 (6%)	215 (36%)	20 (3%)
Nausea and vomiting	198 (32%)	17 (3%)	193 (32%)	4 (<1%)
Stomatitis and ulceration	345 (57%)	89 (15%)	125 (21%)	7 (1%)
Perforation	16 (3%)	16 (3%)	1 (<1%)	1 (<1%)
Fistula	14 (2%)	5 (<1%)	1 (<1%)	0
Haemorrhagic events	270 (44%)	32 (5%)	142 (24%)	10 (2%)
Epistaxis	208 (34%)	14 (2%)	56 (9%)	0
Gastrointestinal haemorrhage	62 (10%)	12 (2%)	34(6%)	6 (1%)
Vascular disorders	264 (43%)	97 (16%)	142 (24%)	43 (7%)
Hypertension	218 (36%)	81 (13%)	69 (12%)	20 (3%)
Venous thrombotic events	23 (4%)	21 (3%)	36 (6%)	35 (6%)
Arterial thrombotic events	12 (2%)	7 (1%)	18 (3%)	15 (3%)
Asthenia or fatigue	376 (62%)	97 (16%)	349 (58%)	46 (8%)
Infection	338 (55%)	123 (20%)	251 (42%)	60 (10%)
Upper respiratory tract infections	97 (16%)	4 (<1%)	84 (14%)	3 (<1%)
Fever	68 (11%)	5 (<1%)	58 (10%)	1 (<1%)
Neutropenic complications	92 (15%)	87 (14%)	42 (7%)	41 (7%)
Oedema	69 (11%)	3 (<1%)	170 (28%)	5 (<1%)
Alopecia	224 (37%)	0	269 (45%)	0
Nail changes	156 (26%)	14 (2%)	153 (26%)	2 (<1%)
Nervous system	320 (52%)	58 (10%)	325 (54%)	56 (9%)
Headache	97 (16%)	4 (<1%)	45 (8%)	0
Peripheral neuropathy	111 (18%)	14 (2%)	156 (26%)	16 (3%)
Respiratory	409 (67%)	65 (11%)	236 (40%)	24 (4%)
Dysphonia	230 (38%)	3 (<1%)	36 (6%)	0
Breathing abnormalities	103 (17%)	14 (2%)	76 (13%)	4 (<1%)
Cough	116 (19%)	4 (<1%)	85 (14%)	0
Musculoskeletal	229 (38%)	27 (4%)	286 (48%)	37 (6%)
Appetite disorders	192 (31%)	12 (2%)	111 (19%)	5 (<1%)
Eye problems	163 (27%)	7 (1%)	108 (18%)	3 (<1%)
Proteinuria	275 (45%)	38 (7%)	214 (36%)	7 (1%)

Data are number of patients (%). We included all side-effects for which the frequency of grade 3–4 events was greater than 5% in either group, and those expected with inhibitors of angiogenesis.

Table 5: Treatment-emergent adverse events

Panel: Research in context

Systematic review

We did a systematic review of PubMed using the search terms "docetaxel", "metastatic", "prostate cancer", and "randomized trial". We identified two phase 3 trials: TAX327 and SWOG 99-16. Findings from these trials showed improved survival in men with metastatic castration-resistant prostate cancer who received chemotherapy with docetaxel and prednisone (TAX327) or docetaxel and estramustine (SWOG 99-16), compared with the previous approved standard of mitoxantrone and prednisone; docetaxel given intravenously every 3 weeks with daily oral prednisone or prednisolone (approved as standard first-line chemotherapy for metastatic castrate-resistant prostate cancer). A systematic review in February, 2013, of PubMed, Google, and meeting reports of ASCO and ESMO using the same search terms identified seven large phase 3 trials that attempted, unsuccessfully, to improve overall survival in men with castrate-resistant prostate cancer by combining docetaxel with a targeted agent. One of these negative trials assessed bevacizumab, an anti-angiogenic agent that inhibits VEGF.

Interpretation

Our findings showed that aflibercept, an inhibitor of angiogenesis that inhibits a broader spectrum of angiogenic growth factors than bevacizumab, did not increase overall survival in men with metastatic castrate-resistant prostate cancer when combined with docetaxel and prednisone, but increased toxicity. The standard first-line chemotherapy treatment for such men remains docetaxel with prednisone or prednisolone.

Margaret Cancer Centre (Toronto, Canada), median survival was longer for men treated as part of a clinical trial,[27] suggesting that the median survival of men in the VENICE trial is probably longer than would be achieved in general oncological practice.

There were no differences in secondary time-to-event endpoints, including time to first skeletal-related event and progression-free survival, between the study groups. However, in patients treated with aflibercept, we detected more tumour responses than in those given placebo, and although not statistically significant, PSA response was greater with aflibercept than it was with placebo. Aflibercept added toxicity and led to more study discontinuations than placebo. The data have not been analysed to compare the proportion of men who had predefined levels of improvement in health-related quality of life in the two groups of the study, and this will be reported subsequently. However, that the addition of aflibercept to docetaxel and prednisone would improve health-related quality of life seems unlikely in view of the greater toxicity with aflibercept.

Both the primary tumour and metastases from prostate (and other) cancers require angiogenesis to provide nutrients and thereby allow cancer cells to survive and proliferate. Aflibercept inhibits a broader spectrum of angiogenic growth factors (VEGF-A, VEGF-B, PlGF1, and PlGF2)[16,17] than does bevacizumab, and we expected that this broader spectrum would lead to more effective inhibition of angiogenesis and improved efficacy compared with placebo. The greater number of tumour responses and non-statistically significantly higher PSA response in the aflibercept group suggest that aflibercept has biological activity, and appropriate biomarkers could identify subpopulations that would benefit from treatment. Potential relations between putative biomarkers and efficacy or safety will be assessed by the investigators; however, concentrations of VEGF have not been

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associated with efficacy in trials assessing bevacizumab.[28] Many growth factors stimulate angiogenesis, and activation of alternate angiogenic pathways is a potential cause of resistance to VEGF inhibitors. In attempts to inhibit multiple points of the angiogenic pathways simultaneously, receptor tyrosine kinase inhibitors in combination with bevacizumab have been studied, but have resulted in additional toxicity without therapeutic benefit.[29]

The additional toxicity seen when aflibercept was added to docetaxel led to a higher proportion of patients completing fewer than four treatment cycles (22% *vs* 10%) and to more frequent discontinuation of study treatment (44% *vs* 21%) than with placebo. The shorter treatment duration might have prevented differences in potential anti-tumour activity from translating into improvements in overall survival and other time-to-event endpoints. The proportion of fatal adverse events that were probably due to toxicity of treatment was 3·4% in the aflibercept group and 1·5% in the placebo group. The number of toxic deaths in the control group was slightly higher than in previous phase 3 studies, in which the incidence of fatal adverse events varied between 0% and 1·2% for patients receiving docetaxel and prednisone,[3,8,13] and was mainly due to infection. The occurrence of fatal toxic events in the aflibercept group was similar to the 4% noted for men receiving docetaxel and bevacizumab in the only other study that assessed an inhibitor of angiogenesis, CALGB 90401,[13] and the types of grade 3–4 toxicity in the aflibercept group were similar to those reported for bevacizumab. The age distribution of patients treated in these studies is similar, although in the present study men with a fatal adverse event were older and more likely to have a previous cardiovascular risk factor than were those who did not have a fatal adverse event.

Aflibercept has been assessed with standard chemotherapy in other large placebo-controlled phase 3 clinical trials for metastatic colorectal, pancreatic, and lung cancer.[30–32] Only the trial in second-line metastatic colorectal cancer, in which aflibercept was used in combination with FOLFIRI (the VELOUR study[30]) resulted improved overall survival (a median of about 6 weeks), which led to approval of aflibercept by the US Food and Drug Administration for this indication.

Nine large phase 3 trials (including this trial) have now assessed docetaxel and prednisone with or without a targeted agent for men with metastatic castrate-resistant prostate cancer, and findings from the eight reported trials (findings from the SYNERGY trial are awaited; NCT01188187) have shown no difference in survival and increased toxicity in most of the experimental groups (table 6).[8–15] Together, these eight trials recruited 7800 men and probably cost close to US$1 billion. The VENICE trial was based on minimal preclinical and early clinical data, and the decision to proceed rapidly to phase 3 was based on the expectation that anti-angiogenic agents would be effective and that docetaxel, prednisone, and bevacizumab would become the new standard of care. Several of the other trials of docetaxel and prednisone were based on minimal preclinical or early clinical data.[10–15] Prostate cancer investigators and sponsors should learn from this experience: future large trials should proceed only after rigorous preclinical and phase 2 data show substantial preliminary evidence of benefit, and they should have tighter criteria for early stopping if substantial benefit can be excluded at interim analyses.

	Number of participants	Partner drug	Result
ASCENT II[8]	953	DN101 (calcitriol)	Poorer survival in experimental group versus control Increased toxicity
VITAL II[10]	408	GVAX vaccine	Poorer survival in experimental group versus control
SWOG S0421[11]	991	Atrasentan	No difference in PFS or survival
ENTHUSE[12]	594	Zibotentan	No between-group difference in survival Increased toxicity
CALGB 90401[13]	1050	Bevacizumab	No between-group difference in survival (better PFS) Increased toxicity
MAINSAIL[14]	1059	Lenalidomide	No between-group difference in survival Increased toxicity
READY[15]	1522	Dasatinib	No between-group difference in survival or other endpoints Increased toxicity
VENICE (present study)	1224	Aflibercept (VEGF-Trap)	No between-group difference in survival Increased toxicity
SYNERGY*		Custirsen (OGX-011)	Study not completed (no data available)

PFS=progression-free survival. *NCT01188187

Table 6: **Trials comparing docetaxel plus prednisone alone or with a targeted agent in men with metastatic castrate-resistant prostate cancer**

Contributors

IFT (study chair) and MR, BT, and DP (cochairs), along with Sanofi employees (including EM and LH) contributed to the study design, study conduct, and study follow-up to the final analysis. Sanofi employees (including EM and LH) did the interim and final analyses and provided interpretation of the results. The detailed clinical study report was drafted primarily by the sponsor (including EM) and made available to IFT, who approved it. IFT drafted the paper, and contributed to subsequent drafts with all authors. IFT, KF, SI, CTK, AF, IS, FO, GG, VM, SB, TG, LV, OA, AK, TE, AB, BT, and MR were investigators in the study and contributed to the recruitment, treatment of the patients, and collection of the data.

Conflicts of interest

Sanofi funded this trial. KF, AF, IS, FO, GG, SB, TE, AB, DP, BT, and MR have acted as paid consultants to Sanofi. IFT has received research funding from Sanofi. EM and LH are employees of Sanofi and hold stock in the company. SI, CTK, VM, TG, LV, OA, and OK declare that they have no conflicts of interest.

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REVIEW

Complementary actions of VEGF and Angiopoietin-1 on blood vessel growth and leakage*

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Abstract

Vascular endothelial growth factor (VEGF) and Angiopoietins are families of vascular-specific growth factors that regulate blood vessel growth, maturation and function. To learn more about the effects of these factors *in vivo*, we have overexpressed VEGF-A or Angiopoietin-1 (Ang1) in two systems in mice, and examined the effects on blood vessel growth and function. In one set of studies, VEGF, Ang1, or both factors, were transgenically overexpressed in the skin under the keratin-14 (K14) promoter. The skin of mice overexpressing VEGF (K14-VEGF) had numerous tortuous, capillary-sized vessels which were leaky to the plasma tracer Evans blue under baseline conditions. In contrast, the skin of mice overexpressing Ang1 (K14-Ang1) had enlarged dermal vessels without a significant increase in vessel number. These enlarged vessels were less leaky than those of wild-type mice in response to inflammatory stimuli. In double transgenic mice overexpressing VEGF and Ang1, the size and number of skin vessels were both increased; however, the vessels were not leaky. In a second set of studies, VEGF or Ang1 was systemically delivered using an adenoviral approach. Intravenous injection of adenovirus encoding VEGF (Adeno-VEGF) resulted in widespread tissue oedema within 1–2 days after administration, whereas injection of Adeno-Ang1 resulted in the skin vessels becoming less leaky in response to topical inflammatory stimuli or local injection of VEGF. The decreased leakage was not accompanied by morphological changes. Thus, overexpressing VEGF appears to promote growth of new vessels accompanied by plasma leakage, whereas overexpressing Ang1 promotes the enlargement of existing vessels and a resistance to leakage. Further understanding of the interrelationship of these factors during normal development could lead to their application in the treatment of ischaemic diseases.

Key words adenovirus; angiogenesis; inflammation; Tie-2 receptor; transgenic mice; vascular endothelial growth factor; vascular leakage.

Introduction

Two families of endothelial-specific growth factors, vascular endothelial growth factors and Angiopoietins, are necessary for the formation of blood vessels. These factors seem to act in co-ordinated and complementary ways to produce mature blood vessels. Vascular endothelial growth factor A (VEGF-A, here called VEGF), the initial member of the VEGF family to be identified (Ferrara et al. 1991; Dvorak et al. 1992), is essential for early blood vessel formation and angiogenesis. Mice deficient in even one allele for VEGF die in embryogenesis due to a decrease in endothelial cell number and severe defects in blood vessel formation (Carmeliet et al. 1996; Ferrara et al. 1996). Angiopoietin-1, the first member of a second family of endothelial-specific factors (Davis et al. 1996; Maisonpierre et al. 1997; Valenzuela et al. 1999), is essential for a later stage of blood vessel formation. Mice deficient for Ang1 die by embryonic day 12.5 (E12.5) due to defects in vessel remodelling and maturation (Suri et al. 1996). VEGF and Ang1 act via distinct endothelial-cell-specific

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tyrosine kinase receptors, which are also essential for embryogenesis and blood vessel formation. Deletion of the VEGF receptor VEGF-R2 (Fong et al. 1995; Shalaby et al. 1995) or the Angiopoietin receptor (Tie-2) (Dumont et al. 1994; Sato et al. 1995) is also lethal to the embryo, with phenotypes broadly similar to those in the corresponding ligand-deficient mice.

Thus a model has evolved to describe the role of these factors in developmental angiogenesis. In particular, VEGF-A and its endothelial cell receptor VEGF-R2 are believed to play a role in vasculogenesis and early angiogenesis, whereas Ang1 and its receptor are believed to be involved in blood vessel remodelling and maturation (Hanahan, 1997; Yancopoulos et al. 2000). However, because of the early lethality of the gene-targeted embryos, it has been difficult to fully define the role of these factors in adult and pathological angiogenesis.

To gain further insight into the function of VEGF and Ang1 *in vivo*, we have used two approaches to overexpress these factors in mice. In particular, we have used tissue-specific transgenic mice and adenoviral vectors. In the transgenic approach, VEGF or Ang1 was overexpressed continuously in the mouse skin from mid-embryonic stages into adulthood. The factors, and their effects, were localized to the skin (Fig. 1A). In the adenoviral approach, VEGF or Ang1 was expressed systemically in otherwise normal adult mice, and the factors acted for a defined duration on blood vessels throughout the mouse (Fig. 1B). Comparing and contrasting these different approaches and the different factors has helped reveal the distinct actions of VEGF and Ang1 on blood vessel growth and leakage.

Transgenic overexpression – K14-VEGF and K14-Ang1 mice

Several groups have generated mice which overexpress VEGF-A in the skin, under either K14 or K5 promoters (Detmar et al. 1998; Larcher et al. 1998; Thurston et al. 1999). We generated K14-$VEGF_{164}$ transgenic mice (Thurston et al. 1999), which appeared normal but had some redness in the skin of the ears and snout. The epidermis of the K14-VEGF mice was thickened, and the dermis contained infiltrating leucocytes. Lesions appeared in the ear skin of older mice (Thurston et al.

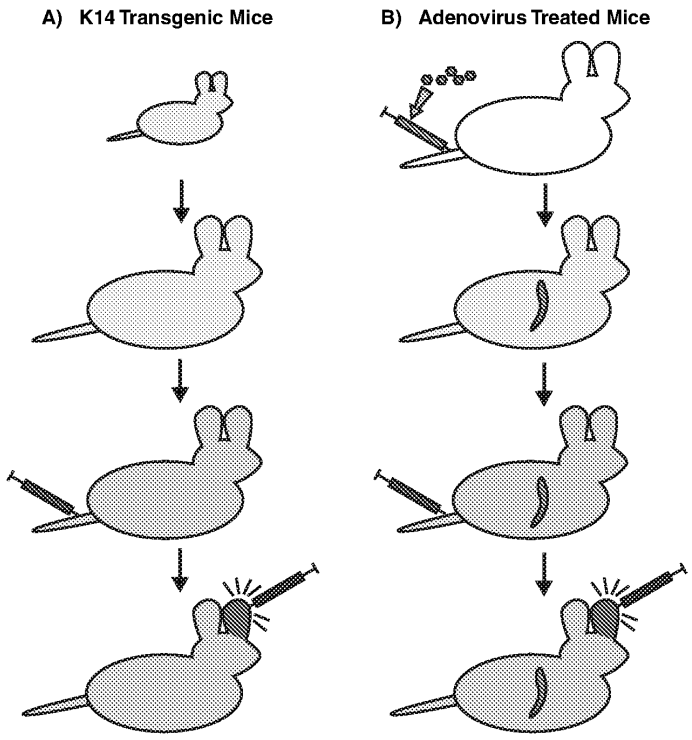


Fig. 1 Outline of Evans blue leakage experiments in (A) K14 transgenic mice and (B) adenovirus treated mice. (A) In transgenic mice, secreted factor (VEGF or Ang1 – green) is overexpressed in embryonic skin and throughout the lifetime of the mouse. Evans blue dye (blue) was injected into adult mice, and then inflammatory stimuli (red) were applied topically to ear skin on one side (other ear served as control). (B) In adenovirus experiments, adenovirus encoding factor (VEGF or Ang1 – green hexagons) was injected intravenously into normal adult mice (white). Factor was overexpressed in liver and secreted into circulation. Inflammatory stimuli were applied topically to the distal site: the ear skin on one side.

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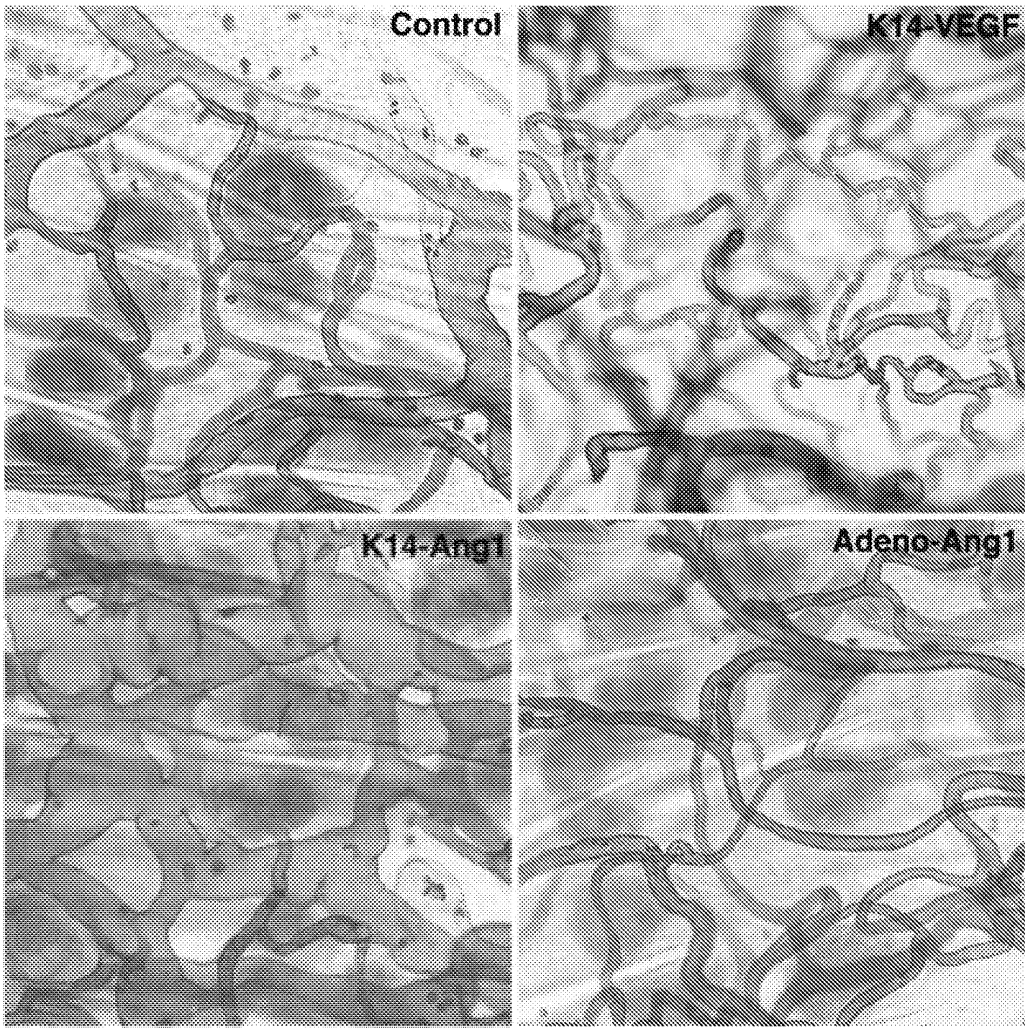


Fig. 2 Morphology of blood vessels in ear skin of control, K14-VEGF, K14-Ang1, and Adeno-Ang1 mice. Vessels were stained by intravascular perfusion of biotinylated *Lycopersicon esculentum* lectin and DAB-peroxidase reaction, and examined in whole mounts (Thurston et al. 1999, 2000). Lectin binds to the luminal surface of endothelium and reveals blood vessels.

1999). Upon examination of tissue sections stained for endothelial cells with antibodies to platelet endothelial cell adhesion molecule (PECAM, CD31) or whole mounts stained with intravascular lectin, the skin of K14-VEGF mice showed increased blood vessel density characterized by increased numbers of small capillary-sized vessels near the epidermis and surrounding the hair follicles (Fig. 2). The tortuous skin vessels of K14-VEGF mice showed leakage of the plasma tracer Evans blue under baseline conditions (Thurston et al. 1999). In addition, the basement membrane of these vessels could be labelled by intravascular perfusion of Ricin lectin, indicating defects in the endothelial barrier. Application of inflammatory stimuli to the ear skin resulted in even larger amounts of plasma leakage. The VEGF transgenic mice highlight the role of VEGF as a potent angiogenic factor, but also emphasize that the resultant vessels can be leaky and inflamed.

Mice overexpressing Ang1 under the K14 promoter were also produced (Suri et al. 1998). The skin of K14-Ang1 mice was notably reddened, but the epidermis was normal in thickness and the dermis did not contain

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infiltrating cells. The dermal vessels were dramatically increased in diameter compared to control mice (Fig. 2), but only moderately increased in number. The enlarged vessels were in the position of capillaries subjacent to the epidermis and surrounding the hair follicles. The enlarged vessels had an increased number of endothelial cells, indicating that Ang1 increased endothelial cell proliferation or survival (Thurston et al. 1999). Unlike K14-VEGF mice, the vessels in K14-Ang1 mice were not leaky under baseline conditions and, remarkably, seemed to be resistant to plasma leakage induced by inflammatory mediators such as histamine, serotonin and mustard oil.

K14-Ang1 mice were bred to K14-VEGF mice (Thurston et al. 1999). The skin of the resultant double transgenic K14-Ang1/VEGF mice was dramatically reddened, and the vascularity of the skin was higher than either K14-VEGF or K14-Ang1 mice. The morphology of the dermal vessels appeared to be a combination of the Ang1 and VEGF effects. In particular, numerous small vessels and enlarged vessels were both present (Thurston et al. 1999). The dermis of K14-Ang1/VEGF mice was normal in thickness and did not contain infiltrating leucocytes. Furthermore, the skin vessels in K14-Ang1/VEGF mice were not leaky to Evans blue or Ricin lectin under baseline conditions (Thurston et al. 1999). Thus, Ang1 seems to inhibit some of the inflammatory actions of VEGF, but Ang1 and VEGF appear to act on distinct signalling pathways for vessel growth.

Adenoviral overexpression – Adeno-VEGF and Adeno-Ang1

Adenoviruses encoding $VEGF_{164}$, Ang1, or green fluorescent protein (GFP) as a control, driven by the cytomegalous viral (CMV) promoter were injected intravenously into mice (age 8–10 weeks) (Thurston et al. 2000). GFP was localized in the liver, confirming that most (> 95%) of the adenoviral gene expression is in hepatocytes (Yao et al. 1996; Michou et al. 1997). High levels of VEGF or Ang1 (10 $\mu g\ mL^{-1}$) were detected in the serum within 1 day after injection of adenovirus and, depending upon the strain of mouse, the levels remained above 500 $ng\ mL^{-1}$ for 10 days or longer.

Following injection of adeno-VEGF (1×10^8 pfu or more), mice became lethargic and died within 2–3 days. Histological examination of various organs in the mice given adeno-VEGF revealed evidence of widespread oedema (Thurston et al. 2000). In contrast, mice injected with adeno-Ang1 (1×10^9 pfu) appeared healthy and remained active. Similar to the K14-Ang1 mice, the blood vessels in the skin of mice given adeno-Ang1 became resistant to the plasma leakage normally induced by local injection of VEGF or topical application of mustard oil (Thurston et al. 2000). The resistance to leakage was found at 1 day after intravenous injection of adenovirus. However, unlike the K14-Ang1 mice, adult mice given adeno-Ang1 did not appear reddened, and the morphology of the skin blood vessels was normal for at least 7 days after adenoviral injection (Fig. 2) (Thurston et al. 2000). Subsequent experiments have shown that the antileakage action can be duplicated by intraperitoneal injection of Ang1 proteins (E. Joffe et al. unpublished results); thus this effect is not due to the adenoviral production of Ang1.

Discussion

Our experiments, using two approaches to overexpress VEGF and Ang1, demonstrate that these factors act separately and distinctly on blood vessels. In both overexpression systems, Ang1 resulted in vessel enlargement and resistance to leak, whereas VEGF resulted in leakiness and, if expressed locally, in vessel sprouting. The actions on blood vessel growth appear to be able to take place independently because, when given together, VEGF caused vessel sprouting and Ang1 caused vessel enlargement. However, at least in the situation where the two factors are overexpressed transgenically, the antileakage action of Ang1 appears to predominate.

In contrast to the transgenic mice in which Ang1 was overexpressed throughout development, Ang1 given to adults did not cause vessel enlargement in the skin, even when given for 50 days. Why did adenoviral expression of Ang1 not cause vessel enlargement? One possibility is that the vasculature in adults is less plastic than in embryonic and neonatal mice, and thus does not enlarge in response to Ang1. Another possibility is that Ang1 acts differently depending on whether it is delivered via the circulation or locally in the interstitium. Studies in which neonatal mice are treated with Ang1, and those in which Ang1 is inducibly overexpressed in adult mice using inducible transgenic approaches, may help shed light on this question.

Our findings using overexpression systems support and extend the studies of gene-targeted mice. Both sets of studies show clearly that VEGF and Ang1 play

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distinct and complementary roles in blood vessel development. Our overexpression studies help to pinpoint the distinctions, in particular highlighting the vessel enlargement and maturation actions of Ang1, and the vessel sprouting and leakiness actions of VEGF. However, it is not known whether the actions of these factors that were highlighted in our experimental overexpression systems are indeed analogous to their roles in normal development. For example, does VEGF induce leakage during normal blood vessel development? Does Ang1 have an antileakage action in development? Does coexpression of Ang1 normally prevent VEGF-induced leakage? Does Ang1 help vessels to enlarge in development? It must be borne in mind that the readouts from the assays used in experimental studies may not have a direct correlate in normal blood vessel growth. Nevertheless, our studies help define the range of actions that can be induced by endothelial-specific factors.

One clinical application of Ang1 may be to use it in combination with VEGF to grow normal, non-leaky vessels. Therapeutic application of VEGF has been tested in a number of situations to induce the growth of new blood vessels (therapeutic angiogenesis – Isner & Losordo, 1999). However, a potential side-effect of large amounts of VEGF may be the growth of leaky vessels. Indeed, some reports have suggested this may be the case (Baumgartner et al. 1998; Springer et al. 1998; Lee et al. 2000). Co-administration of Ang1 may help reduce the leak-inducing actions of VEGF without suppressing the vessel growth actions. Further studies comparing the actions of VEGF and Ang1 will undoubtedly help define the discrete steps of normal blood vessel development.

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Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis

Yu-Ping Xia, Baosheng Li, Donna Hylton, Michael Detmar, George D. Yancopoulos, and John S. Rudge

Gene therapy approaches involving vascular endothelial growth factor (VEGF) to promote therapeutic angiogenesis are under consideration for conditions ranging from ischemic heart disease to nonhealing skin ulcers. Here we make the surprising observation that the transgenic delivery of VEGF to the skin results in a profound inflammatory skin condition with many of the cellular and molecular features of psoriasis, including the characteristic vascular changes, epidermal alterations, and inflammatory infiltrates. Even longstanding psoriatic disease remains dependent on the transgenic VEGF in this model because it can be effectively reversed by the addition of VEGF Trap, a potent VEGF antagonist. Previous attempts to faithfully replicate the psoriatic phenotype through the transgenic delivery of epidermal keratinocyte growth factors or inflammatory mediators generated phenotypes with only partial resemblance to human psoriasis, leaving unanswered questions about the etiology of this disease. The ability of transgenic VEGF to induce a psoriasiform phenotype suggests a new etiology and treatment approach for this disease and further substantiates emerging concerns about possible proinflammatory adverse effects that might be associated with therapeutic attempts to deliver VEGF. (Blood. 2003;102:161-168)

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Introduction

Vascular endothelial growth factor (VEGF) is a potent mediator of angiogenesis, prompting recent efforts to therapeutically exploit this factor in conditions involving pathologically decreased blood flow, such as ischemic heart disease and nonhealing skin ulcers. Some recent studies,[1-6] however, have raised concerns about whether the delivery of VEGF could also have deleterious consequences. Here we make the surprising observation that chronic transgenic delivery of VEGF to the skin can result in a profound inflammatory condition with many of the cellular and molecular hallmarks of human psoriasis, such as hyperplastic and inflamed dermal blood vessels,[7] epidermal thickening (termed acanthosis) with aberrant keratinocyte differentiation,[8] and characteristic inflammatory infiltrates.[9,10]

It has long been known that the reddened appearance of psoriatic skin is caused by hyperplastic dermal blood vessels, that vascular changes occur early in this disease, and that levels of VEGF are elevated in psoriatic skin.[7,11-13] In addition, the dermal vessels in psoriasis appear to be highly abnormal in that they are hyperpermeable, contributing to the edema that characterizes psoriatic skin, and in that they express markers of an inflamed vasculature, such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intracellular adhesion molecule-1 (ICAM-1).[14-16] In addition, the levels of soluble adhesion molecules in the sera of psoriasis patients, particularly E-selectin, provide a valuable surrogate marker of disease severity and therapeutic efficacy of various treatments.[17-20] Despite this, most efforts at understanding the etiology of this disease have focused on the epidermal and inflammatory aberrations. In the abnormally thickened epidermis, the top layer (termed stratum corneum), usually consisting of cornified keratinocytes lacking nuclei, instead contains cells with nuclei (termed parakeratosis). Furthermore, this keratinized upper layer is excessively thickened (termed hyperkeratosis). Most striking, the epidermis produces highly abnormal and characteristic fingerlike projections into the underlying dermis, termed rete ridges. The typical inflammatory cell infiltrate seen in psoriasis is composed of epidermal microabscesses, increased numbers of mast cells, neutrophils and macrophages in the dermis, and activated T cells in the dermis and epidermis.[21,22] T-cell subsets achieve a unique distribution as psoriasis evolves, with $CD4^+$ T cells congregating primarily in the dermis while $CD8^+$ T cells migrate from their normal dermal position to the epidermis, which is usually free of leukocytes.[23,24]

It is clear that psoriatic skin is a hotbed of epidermal growth factors and inflammatory mediators.[23,25-30] Supportive evidence of a key role for such mediators comes from patients who respond to immunosuppressive, anti-inflammatory, and antiproliferative therapies such as cyclosporine, methotrexate, tacrolimus, corticosteroids, and ultraviolet-light–activated psoralen. However, extensive efforts aimed at transgenically delivering inflammatory mediators or keratinocyte growth factors to the skin have not completely reproduced the psoriatic phenotype[28,31-36] (Figure 1), which has thus far only been faithfully modeled in animals by transplanting human psoriatic skin onto mice with severe combined immunodeficiency disease (SCID)[10,23] (Figure 1). Factors such as keratinocyte growth factor, transforming growth factor-α (TGF-α), and interleukin-20 (IL-20) promote some degree of epidermal hyperplasia, in certain cases with associated inflammation, but fail to produce many of the hallmarks of human psoriasis.[28,34,37,38] Transgenic

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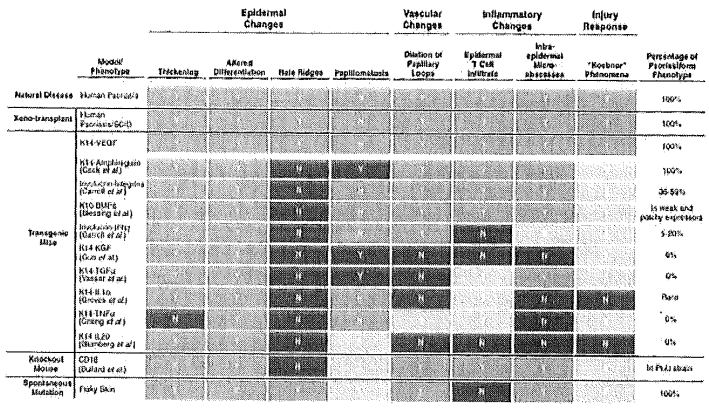


Figure 1. Summary of various mouse models and their resemblance to human psoriasis. The top line indicates whether characteristic changes are seen in human psoriasis (Y for yes, N for no). Other models are compared with the human standard and are blocked in green if they match human psoriasis or in red if they do not match. Question marks and the yellow blocks indicate that the feature in question was not examined. Note that only 2 models precisely match human psoriasis in all the features indicated here—a xenotransplantation model in which human psoriatic skin was transplanted onto a SCID mouse (second line) and the K14VEGF transgenic mouse discussed (third line).

delivery of amphiregulin has resulted in the most promising transgenic model, but this model still lacks the characteristic rete ridge projections seen in human psoriasis, and it is also prone to papillomatosis, which is not typical in the human disease[29] (Figure 1). In addition, a chronic inflammatory skin condition developed in mice in which CD18 was knocked out, but only when the CD18-deficient 129/Sv mice were backcrossed onto the PL/J strain.[39] Again, these mice lacked the rete ridge structures that are highly characteristic of human psoriasis (Figure 1).

The problems with the above transgenic models of psoriasis raise the possibility that there is an upstream predisposition for psoriasis that can somehow be triggered so as to lead to the extremely diverse cytokine and growth factor abnormalities that drive psoriasis and that none of these individual downstream mediators is sufficient to induce the full spectrum of psoriatic disease. Consistent with the notion that psoriasis involves an underlying predisposition, the wounding of asymptomatic skin in psoriatic patients can trigger a complete psoriatic response adjacent to the wound, in a classic reaction termed the Koebner phenomenon.[40]

Our studies suggest that excess VEGF may provide just such a predisposition by inducing a vascular inflammatory response that then predisposes to more widespread tissue inflammation closely resembling the psoriatic state. We report that young mice transgenically overexpressing VEGF in the skin initially lack overt disease but have a predisposition such that wounding can elicit the psoriatic phenotype, analogous to the Koebner phenomenon in humans. In older transgenic mice, the condition progresses until a profound inflammatory skin condition spontaneously develops with many of the cellular and molecular hallmarks of psoriasis, from characteristic epidermal alterations including dramatic rete ridge formation to the inflammatory infiltrates typical of psoriasis. Even late-stage disease remains dependent on transgenic VEGF because it can be effectively reversed by the addition of a potent VEGF antagonist. The ability of transgenic VEGF to induce a psoriasiform phenotype suggests a new etiology and treatment approach for this disease and further substantiates emerging concerns[6] about possible proinflammatory adverse effects that might be associated with therapeutic attempts to deliver VEGF.

Materials and methods

K14-VEGF transgenic mice

A keratin-14 (K14)–based expression vector and a mouse cDNA encoding $VEGF_{164}$ were used to generate K14-VEGF transgenic mice on the *FVB* genetic background, as previously described[4]; mice homozygous for this transgene were used throughout the studies described here. All animals in the facility are cared for in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996).

Tissue processing and immunostaining

Tissue from the K14-VEGF transgenic and wild-type littermate mice used in these studies was matched according to sex, age, and wound site. Fixed sections were immunostained with antimouse platelet-endothelial cell adhesion molecule-1 (PECAM-1) (CD31; BD PharMingen, San Diego, CA), antimouse CD4 (BD PharMingen), antimouse CD8 (BD PharMingen), antimouse F4/80 (Serotec, Oxford, England), or antimouse VEGF (R&D Systems, Minneapolis, MN) following the manufacturer's instructions. Stainings for keratinocyte proliferation and differentiation markers or leukocyte adhesion molecules were performed as previously described[41] with rabbit polyclonal antibody against mouse keratin 6 (K6) (Babco, Richmond, CA) and rat monoclonal antibodies against mouse E-selectin (CD62E), ICAM-1 (CD54), and VCAM-1 (CD106; BD PharMingen) using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA).

Histology

Hematoxylin and eosin (H&E) staining and trichrome staining were performed according to protocols previously described.[42]

Injection of VEGF Trap

VEGF Trap is a fusion of the immunoglobulin 2 domain of human VEGFR1, the immunoglobulin 3 domain of human VEGFR2, and the Fc domain of human immunoglobulin G1 (IgG1), creating a forced homodimer that binds VEGF with high affinity (dissociation equilibrium constant, 1-5 pM) and prolonged in vivo half-life (1-2 days in mice).[43] The 6-month-old K14-VEGF homozygous transgenic mice were treated by the systemic administration of VEGF Trap by subcutaneous injection at a site distant from the psoriatic skin. Mice were treated with either 25 mg/kg VEGF Trap or 12.5 mg/kg human Fc, corresponding to an equal molar concentration as a control, using an injection schedule of every 3 days for 12 days that resulted in a total of 4 injections per animal. Mouse ear tissue was harvested on day 14 for subsequent histologic analyses.

Results

Young K14-VEGF transgenic mice display a mild pre-psoriatic phenotype

As previously reported, K14-VEGF transgenic mice overexpressing VEGF in the epidermis are fertile and overtly healthy.[2,4]

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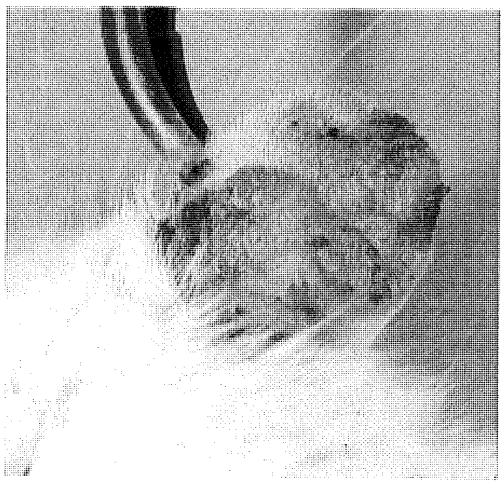


Figure 2. Psoriasiform phenotype. Erythematous, scaly, and thickened skin lesions with associated edema develop in homozygote K14-VEGF transgenic mice older than 5 months.

However, the ear skin of mice homozygous for this transgene is visibly redder than that of their wild-type *FVB* littermates. By 3 months, occasional focal skin lesions begin to develop on the ear and, to a lesser extent, on the dorsal and lateral skin. This condition worsens with age such that pronounced skin lesions are observed on the ears, neck, and snout by 5 months of age, with lesions characterized by erythematous and scaly skin (Figure 2). These lesions coincided with sites of highest expression of the VEGF transgene (data not shown).

An initial histologic screen of the ear skin from young K14-VEGF transgenic mice, at 3 months of age, revealed a mild and potentially pre-psoriatic phenotype in these young mice using standard H&E staining—that is, the epidermis of these mice exhibited moderate acanthosis (epidermal hyperplasia) (Figure 3B, left inset), focal parakeratosis (keratinocytes in the stratum corneum retain nuclei) (Figure 3B, left inset), and mild rete ridge formation on the ventral ear surface (Figure 3B, arrows), compared with age-matched control littermates (Figure 3A). In the dermal compartment, edema contributing to an approximately 2- to 3-fold increase in tissue thickness was observed in the K14-VEGF mice, as was inflammatory cell infiltration in the subepidermal dermis (Figure 3; compare panels A and B).

Consistent with high-level transgenic overexpression of VEGF in the epidermis of these mice, VEGF protein was observed in the epidermis (where it is produced) and on dermal microvessels (where it presumably accumulates after diffusion into the dermis) in patterns (Figure 3A-B; compare right insets), remarkably reminiscent of those seen in human psoriasis.[12]

Young K14-VEGF transgenic mice exhibit a dramatic Koebner-like psoriatic response to injury

In contrast to the mild changes seen under basal conditions, creation of an excisional wound in the dorsal ear skin of 3-month-old K14-VEGF transgenic mice resulted in dramatic invaginations of the epidermis on the ventral side of the ear apposing the wound.

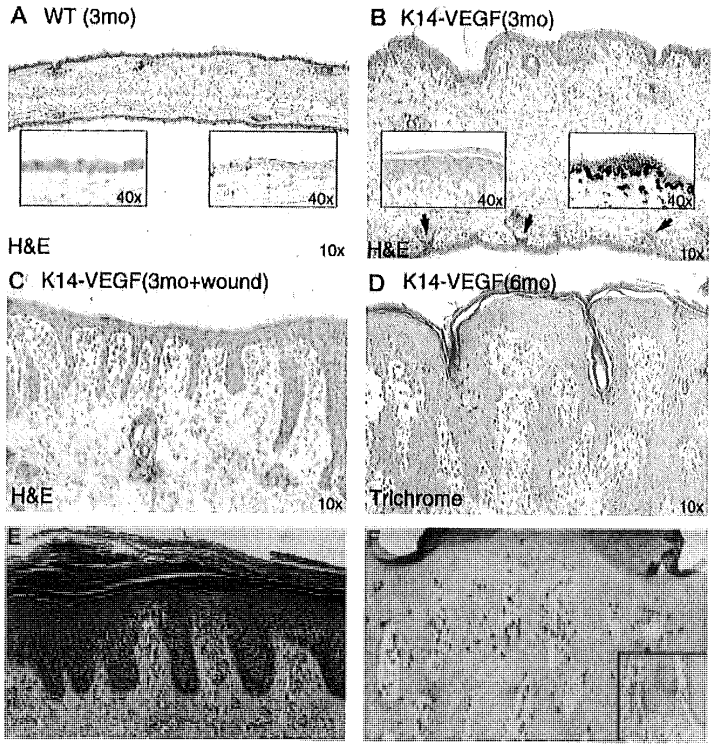


Figure 3. Histologic examination of ear skin from K14-VEGF transgenic mice, using H&E-stained tissue sections. (A) Control, wild-type littermate. Left inset shows epidermis at higher magnification; while right inset shows epidermis is negative for VEGF immunostaining. (B)Transgenic mouse (3 months of age) with edema, mild rete ridge formation on the ventral ear surface (arrows), epidermal acanthosis (left inset), and VEGF immunostaining in epidermis and in dermal microvessels (right inset). (C) Wound-induced rete ridge formation in a 3-month-old VEGF transgenic mouse. (D) Six-month-old VEGF transgenic mice showed spontaneous extensive rete ridge formation and anastomosis. (E) Early-stage human psoriasis shown for comparison. (F) Fully developed human psoriasis shown for comparison. Panels E-F reproduced with permission from Elder et al[48] and Nickoloff and Wrone-Smith,[23] respectively. Original magnifications: × 10 (A-D); and × 40 (insets).

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These invaginations resembled the prominent rete ridge structures present in early human psoriasis (Figure 3E) and were observed in all 10 mouse ears used in the wounding study. Five ears were quantified for rete ridge counts (Figure 3C; Table 1). The induction of rete ridge formation in human pre-psoriatic skin has also been documented after skin injury and is termed the Koebner phenomenon.[40] Accompanying these rete ridge structures were increased dermal cellularity (inflammatory infiltrate), hyperkeratosis, and focal parakeratosis. These data suggest that to induce a severe psoriatic phenotype in the 3-month-old K14-VEGF transgenic animals, it would be necessary to introduce another stimulus such as wounding. This is consistent with the clinical evolution from pre-psoriatic skin to psoriatic lesion in humans (Koebner phenomenon).

Dramatic psoriasiform epidermal lesions spontaneously develop in older K14-VEGF mice

As the K14-VEGF transgenic mice aged, they spontaneously began to develop dramatic lesions resembling full-fledged psoriasis. K14-VEGF transgenic animals older than 5 months of age developed pronounced epidermal rete ridges and marked cutaneous inflammation (Figure 3D). In these lesions, psoriasiform hyperplasia with elongated rete ridges and anastomosis of neighboring rete ridges was found (Figure 3D), revealing a striking resemblance to fully developed psoriasis in humans (Figure 3F). It is important to note that rete ridge formation is one of the most characteristic and longest-recognized histologic features of human psoriasis and that no other transgenic mouse model results in rete ridge formation (Figure 1).

Hyperplastic and inflamed cutaneous blood vessels in K14-VEGF transgenic mice are similar to those observed in human psoriasis

To understand the nature of the visible skin redness in the K14-VEGF transgenic mice, we immunocytochemically stained ear sections with an antibody to an endothelial-cell–specific antigen, PECAM-1. When compared with microvessels in wild-type skin (Figure 4,A) those in 3-month-old K14-VEGF mice with wound-induced psoriasis were obviously dilated and tortuous. The superficial vascular plexus showed that the most prominent angiogenic alterations consisted of vertically oriented vascular tufts in dermal papillae, similar to human psoriasis (Figure 4B). In the unwounded skin of older K14-VEGF mice (6 months of age), these enlarged vessels became more prominent within dermal papillae that were surrounded by a hyperproliferative epidermis undergoing rete ridge anastomosis (Figure 4C). Elongated and enlarged vessels found in the dermal papillae of K14-VEGF mice have a remarkable resemblance to the long, ectatic vessel loops seen in the dermal papillae of human psoriatic skin.

Because the K14-VEGF mice exhibited enlarged and tortuous vessels in dermal papillae analogous to those seen in human psoriatic skin, we next explored whether these hyperplastic vessels also exhibited features of vascular inflammation seen in patients with psoriasis. In particular, the induction of specific endothelial cell adhesion molecules is a hallmark of the hyperplastic and inflamed vessels seen in human psoriatic skin lesions, including the induction of E-selectin (CD62E),[15] VCAM-1 (CD106),[16] and ICAM-1 (CD54).[14] Similar to findings in human psoriasis, the expression of these cell adhesion molecules were prominent in hyperplastic vessels in the psoriasiform skin from K14-VEGF mice (Figure 4F-G).

Table 1. Koebner phenomenon in young K14-VEGF transgenic mice

	Wild-type littermates, wounded	K14-VEGF mice, unwounded	K14-VEGF mice, wounded
Rete ridge counts/unit length of epithelium ± SD, mm	0	2.0 ± 1.3	11.3 ± 5.2

Young is defined as approximately 3 months old.
Each category of littermates consisted of 5 mice.

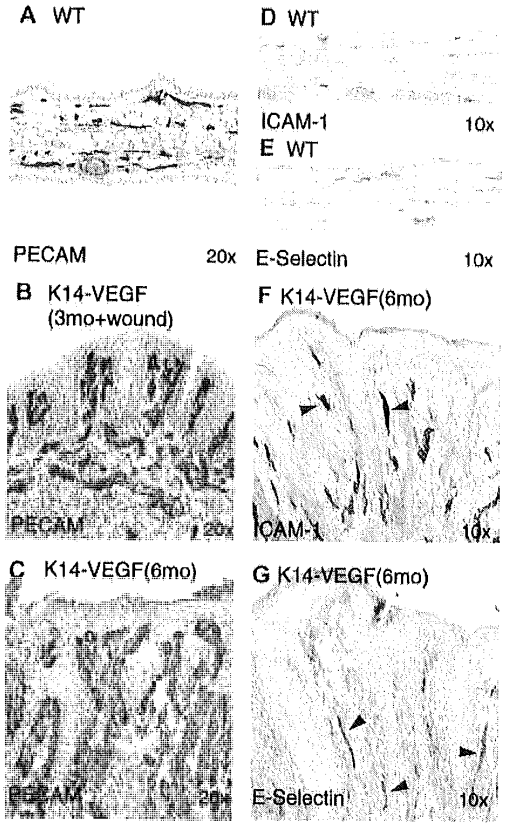


Figure 4. Hyperplastic and inflamed cutaneous blood vessels in K14-VEGF transgenic mice. Immunostaining was performed on cryosections of ear skin from wild-type littermate controls (A, D-E) and transgenic mice (B-C, F-G). PECAM staining showed increased vascular density mostly in the papillary dermis in wound-induced psoriasis in the 3-month-old transgenic mice (B). Enlarged vessels in 6-month-old transgenic mice showed vessels enclosed by anastomosing epidermal rete ridges (C). Immunostaining of E-selectin (G) and ICAM-1 (F) showed positive signals on dermal microvessels in transgenic mice (arrowheads). Original magnifications: × 20 (A-C); and × 10 (D-G).

Abnormal epidermal proliferation and differentiation in K14-VEGF mice resembling that seen in human psoriasis

Epidermal analysis of the psoriasiform lesions in K14-VEGF transgenic mice revealed hyperkeratosis (increased thickness of the stratum corneum) and parakeratosis (retention of nuclei in the cornified keratinocytes) (Figure 5A). Human psoriasis is characterized by similar hyperkeratosis and parakeratosis[44] and by altered epidermal hyperproliferation, as reflected by thickening of the

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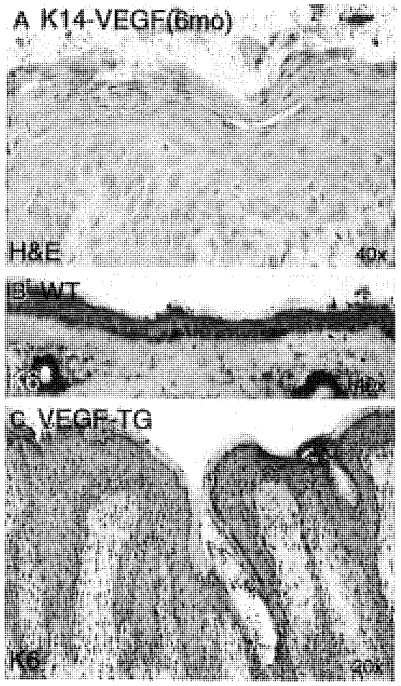


Figure 5. Abnormal epidermal proliferation and differentiation in K14-VEGF transgenic mice. Parakeratosis and hyperkeratosis were observed in H&E-stained skin sections from 6-month-old transgenic mice (A). Immunostaining of keratin K6 showed strong up-regulation throughout the epidermis (compare panels B and C) in 6-month-old transgenic mice.

epidermis and aberrant expression of hyperproliferation-associated keratins K6 and K16 throughout the epidermis, which are normally restricted to sporadic basal keratinocytes and hair follicles.[45] Another similarity we found between the psoriasiform lesions of K14-VEGF mice and human psoriasis was the strong expression of K6 throughout the hyperplastic epidermis of K14-VEGF mice (Figure 5C); normal mouse skin, with the exception of hair follicles and occasional basal keratinocytes, did not express K6 (Figure 5B).

K14-VEGF mice exhibit epidermal microabscesses and inflammatory infiltrates characteristic of human psoriasis

Neutrophil-filled lesions resembling the epidermal microabscesses found in advanced human psoriasis were observed in the epidermis of 6-month-old K14-VEGF transgenic mice (Table 2). One type of microabscess, mimicking the location of Munro microabscesses

Table 2. Quantitation of psoriatic phenotype in older K14-VEGF transgenic mice

	Wild-type littermates	K14-VEGF mice
Mice with lesions, %*	0	98
Intraepidermal $CD8^+$ T-cell counts/unit length of epithelium ± SD, mm†	0	5.55 ± 2.10
Intraepidermal microabscesses per specimen‡	0	4.2

Older is defined as older than 5 months.
*n > 120 mice; †n = 4 mice; ‡n = 5 mice.

described in human psoriasis, was localized within the stratum corneum (Figure 6A),[46,47] and a second type of microabscess, resembling Kogoj microabscesses seen in human psoriasis, was localized immediately beneath the stratum corneum (Figure 6B).[46,47] The presence of microabscesses in human psoriatic skin is a key feature used in the clinical diagnosis of human psoriasis.[46,47]

Analysis of the inflammatory cell infiltrate in 3-month-old K14-VEGF mice revealed a significant increase in both mast cells (as determined by staining for toluidine blue, which stains mast cell granules; Figure 6D) and macrophages (as determined with an antibody to the murine macrophage marker F4/80 antigen (Figure 6G) when compared with wild-type littermate controls (Figure 6C-F). Further increases in mast cells and macrophages were evident as the animals aged to 6 months (Figure 6E-H).

To assess the number and distribution of $CD4^+$ and $CD8^+$ T-lymphocytes, we immunostained for these cells in the 3- and 6-month-old transgenic animals. Results revealed massive infiltration of $CD4^+$ T-lymphocytes localized primarily to the dermis of 3-month- (data not shown) and 6-month-old transgenic mice (Figure 6I). The overall level of $CD8^+$ T-lymphocytes that infiltrated into the transgenic skin was significantly lower than that of

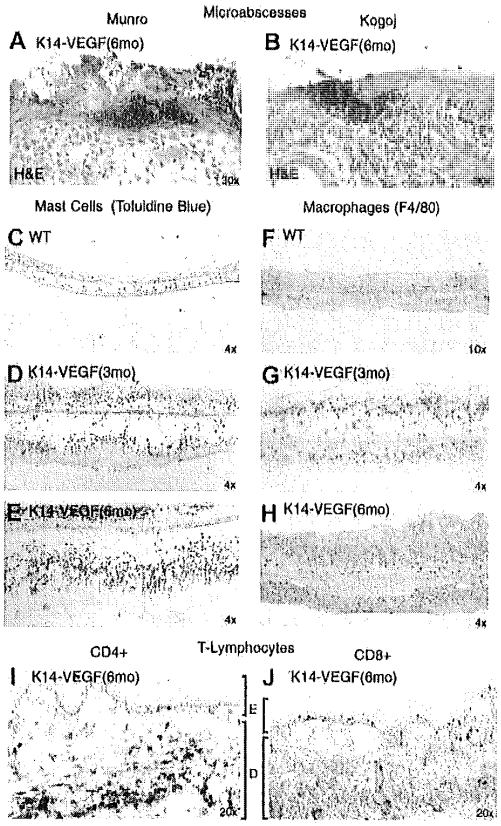


Figure 6. K14-VEGF mice exhibit epidermal microabscesses and inflammatory infiltrates characteristic of human psoriasis. (A) Munro-like microabscess. (B) Kogoj-like microabscesses. Progressive increase of mast cell (C-E) and macrophage (F-H) density with 3-month-old (D, G) and 6-month-old (E, H) transgenic mice compared with wild-type littermates (C, F). $CD4^+$ T-lymphocytes were detected primarily in the dermis (I) and $CD8^+$ T-lymphocytes in the epidermis of 6-month-old transgenic mice (J). E indicates epidermis; D, dermis.

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the $CD4^+$ T-lymphocytes. In young K14-VEGF mice, these $CD8^+$ T-lymphocytes were detected in the dermis and the epidermis (data not shown), whereas the $CD8^+$ lymphocytes translocated and became localized to the epidermis in the 6-month-old transgenic mice (Figure 6J; Table 2). This complementary localization of $CD4^+$ versus $CD8^+$ lymphocytes in dermis and epidermis is also characteristic of human psoriatic skin.

Treatment with VEGF Trap normalizes the psoriatic phenotype in K14-VEGF transgenic mice

Our data demonstrate that many of the histologic and immunologic hallmarks of human psoriasis appear when VEGF is chronically overexpressed in mouse epidermis. To confirm the role of VEGF in the initiation and maintenance of this psoriatic phenotype and to attempt to ameliorate it, we used a potent VEGF inhibitor, VEGF Trap.[43] Six K14-VEGF transgenic mice at 6 months of age with obvious psoriatic lesions were systemically treated with VEGF Trap at a dose of 25 mg/kg every 3 days for 12 days. Although similar lesions in K14-VEGF mice do not regress spontaneously or after control treatments, in 4 of the K14-VEGF mice treated with VEGF Trap, pronounced visual improvement was observed in lesions on gross inspection. The other 2 K14-VEGF mice treated with VEGF Trap displayed moderate improvement. Using enzyme-linked immunosorbent assay (ELISA) to detect antibodies raised against VEGF Trap in these animals, we detected an obvious immune response only in the latter 2 moderate responders, suggesting partial immunoneutralization of VEGF Trap in these mice (data not shown). Histologic evaluation of all 6 K14-VEGF mice treated with VEGF Trap revealed near-complete resolution of the rete ridge elongations (Figure 7A-B), normalization of epidermal architecture and diminution of parakeratosis (Figure 7C-D), and reduction in vascular hyperplasia (Figure 7E-F). In addition, the K6 marker of aberrant epidermal differentiation was normalized by VEGF Trap treatment (Figure 7G-H), as were markers of vascular inflammation E-selectin (Figure 7I-J), ICAM-1 in basal keratinocytes and vasculature (data not shown), and $CD8^+$ T-lymphocyte distribution (Figure 7K-L).

Numerous reports have correlated the level of disease activity in psoriasis with the levels of soluble vascular adhesion molecules in the sera of patients with psoriasis, in particular soluble E-selectin that is presumably cleaved from the surface of inflamed dermal vessels.[17-20] Further correlating our animal model with the human condition, we find that serum levels of E-selectin are much higher in K14-VEGF mice (249.42 ± 38.64 ng/mL) than in control mice (37.34 ± 7.06 ng/mL), and, just as important, these increased levels are reduced with VEGF Trap treatment (to 96.74 ± 16.25 ng/mL). Altogether, our data indicate that VEGF is continuously required to maintain the psoriasiform lesions in older K14-VEGF mice and that even longstanding disease can be dramatically reversed with VEGF blockade.

Discussion

The underlying pathogenic mechanism and the key molecule(s) that are causative for psoriasis have not yet been identified. Recent studies for causative agents have focused on molecular mediators of inflammation or keratinocyte growth. However, attempts to mimic human psoriasis by transgenically overexpressing such mediators in mice do not completely recapitulate the human disease in all its pathologic aspects (Figure 1). In addition, the most

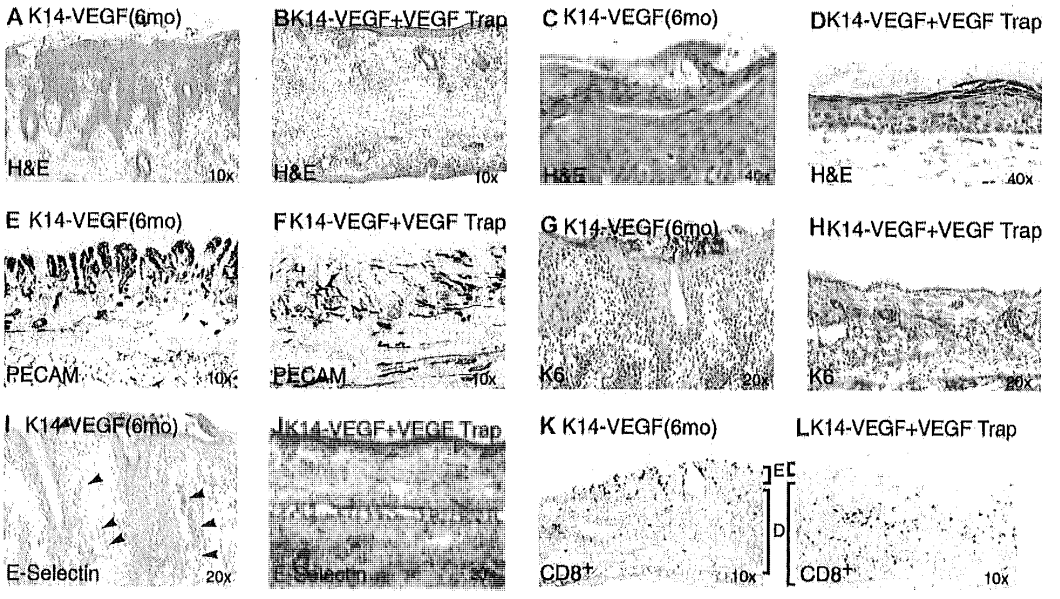


Figure 7. VEGF Trap normalizes the psoriatic phenotype in K14VEGF transgenic mice. Transgenic mice with severe skin lesions were injected with VEGF Trap (25 mg/kg) on days 0, 3, 7, and 12. Tissue was harvested on day 14 for histologic analysis. H&E staining of mouse ear skin treated with VEGF Trap showed clear resolution of rete ridges (compare panels A and B) and decreased parakeratosis/hyperkeratosis (compare panels C and D). Immunostaining with PECAM showed a drop-off of microvessels in the papillary dermis (compare panels E and F). Immunostaining with keratin K6 and E-selectin each showed remarkable down-regulation of signals in the epidermis (compare panels G and H), dermal capillaries (compare panels I and J), respectively. Arrowheads in panel I denote positive staining of blood vessels for E selectin. $CD8^+$ T-lymphocytes shifted localization from the epidermis to the dermis in treated animals (compare panels K and L). E indicates epidermis; D, dermis.

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faithful animal model of psoriasis to date requires the transplantation of human psoriatic skin to SCID mice[23] (Figure 1). Our findings lend credence to earlier suggestions that vascular changes might be among the earliest markers of the human psoriatic state.[7,11] In particular, our studies suggest that VEGF, which has previously been shown to be dramatically elevated in human psoriatic skin,[12] might play a causative role in the vascular changes seen in this disease and also in epidermal and inflammatory alterations. Along these lines, we demonstrate that excess VEGF in the skin is sufficient to create a predisposition to a psoriatic phenotype and that such overexpression eventually leads to the spontaneous development of a psoriasiform condition in mice that recapitulates human psoriasis in many of its features—not only the hyperplastic and inflammatory vascular changes but also the characteristic epidermal alterations and tissue inflammatory cell infiltrates (Figure 1). Additional emerging evidence for a role of VEGF in the etiology of psoriasis comes from recent genetic analyses showing an association between VEGF promoter polymorphisms and the development of psoriatic symptoms (M.D., personal oral communication, November 2002). The induction of psoriasis by VEGF seems to be specific in that skin-specific transgenic delivery of another angiogenic factor, Ang1, does not result in a similar phenotype.[1,4]

In our model, it is clear that excess VEGF does not immediately cause full-blown disease. It takes up to 5 to 6 months for the development of obvious spontaneous disease. Overexpression of VEGF in the adult animal skin by viral gene transfer does not induce psoriasis because short-term VEGF expression is not sufficient to induce the psoriatic phenotype. In fact, it has been shown that the injection of a nonreplicating adenoviral vector, engineered to express $VEGF_{164}$, into the ears of athymic mice only temporarily induced the formation of dilated and leaky angiogenic vessels.[48] Thus, it seems likely that acute overexpression of VEGF creates a dilated, leaky, and inflamed cutaneous vasculature but that chronic overexpression is necessary to yield a more widespread inflammatory condition in the skin with profound epidermal changes resembling psoriasis. Although it is unclear how VEGF results in such widespread changes, it seems likely that the inflamed vasculature, which exhibits elevations in vascular adhesion molecules such as E-selectin, ICAM-1, and VCAM-1, plays a primary role by promoting the extravasation of inflammatory cells to the skin that then lend their own cytokine and chemokine mediators to the process. This inflammatory infiltrate and the tissue edema promoted by the leaky vessels may well compromise the normal barrier function of the skin, allowing the entry of exogenous antigens and further exacerbating the immune state. The creation of a diverse inflammatory milieu may then secondarily lead to epidermal alterations that seem to occur after the initial vascular and inflammatory changes in our model. Regardless of the mechanism by which chronically elevated VEGF in our model results in a psoriasiform phenotype, the maintenance of this abnormal state remains dependent on VEGF—we show that VEGF blockade late in this process can effectively reverse almost all the observed abnormalities. It should perhaps not be surprising, based on previous studies indicating that VEGF can act as a potent and pleiotropic inflammatory agent, that transgenic delivery of VEGF to the skin can lead to a profound inflammatory skin condition. For example, a recent in vitro study using human umbilical vein endothelial cells (HUVECs) showed that VEGF stimulated the expression of ICAM-1, VCAM-1, and E-selectin through nuclear factor-κB activation.[49] VEGF has also been shown to induce monocyte activation and chemotaxis through VEGF receptor-1 (Flt-1), which is expressed on monocytes.[50,51] In addition, VEGF has been shown to induce expression of the chemokine IL-8, which is a potent modulator of the transendothelial migration of neutrophils.[52]

Although we do not yet precisely understand how transgenic overexpression of VEGF eventually leads to a psoriasiform condition in mice, it seems impossible to ignore the possibility that VEGF may play a key causative role in human psoriasis, and it seems important to follow up on this possibility and on the implications for understanding and treating the human disease. Conventional psoriasis treatments that attempt to control the inflammatory response and subsequent epidermal hyperproliferation rely on immunosuppressants and antiproliferative therapy, involving considerable toxicity often without complete resolution. The use of a specific VEGF antagonist, such as VEGF Trap, to eliminate the hyperplastic vascular phenotype, suppress the associated inflammatory state, and reduce the levels of surrogate markers (such as E-selectin) of the disease in human psoriasis may provide a novel therapeutic strategy with minimal adverse side effects. It is also important to note that some existing or emerging therapies for psoriasis may act in part by blocking the VEGF pathway. For example, calcineurin inhibition by cyclosporine or FK-506 may block VEGF production or action,[53,54] and tumor necrosis factor-α (TNF-α) and IL-1 seem to be potent inducers of VEGF. This induction may be important for some of their pathologic actions.[55,56]

Our findings demonstrate that prolonged VEGF overexpression has powerful proinflammatory capabilities in vivo and leads to a skin phenotype resembling human psoriasis. VEGF is likely a key factor in the link between inflammation and angiogenesis. Therefore, its role should be explored in a variety of other inflammatory conditions. Furthermore, our findings substantiate emerging concerns[6] about potential adverse effects that might be associated with therapeutic attempts to chronically deliver VEGF for proangiogenic purposes, particularly with regard to its profound proinflammatory capabilities.

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Electronic Acknowledgement Receipt	
EFS ID:	39027760
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International Application Number:	
Confirmation Number:	8135
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First Named Inventor/Applicant Name:	George D. YANCOPOULOS
Customer Number:	96387
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	0725US06_2020-03-31_Supp_IDS_trans_REGN-008CIPCON5.pdf	50688 e901cdbbb8f3332e5c902149884c35a3bb50ed3d	no	2

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	0725US06__2020-03-31_Supp_IDS_SB08A_REGN-008CIPCON5.pdf	36355 e396b5cc2282672ceee1598c6da7966aa5b2b28b	no	2
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Non Patent Literature	Anti-VEGF_2019.pdf	3877709 38be305da8c8aa0c9220de7e694de59f835d769c	no	10
Warnings:					
Information:					
4	Non Patent Literature	Chatziralli_2018.pdf	1099422 fa8ce24228ee66646858acc7a38e7903fd5b157f	no	7
Warnings:					
Information:					
5	Non Patent Literature	Chung_2013.pdf	218908 041564f7a01739052957d3e9faf294746cc30266	no	10
Warnings:					
Information:					
6	Non Patent Literature	Cooper_1999.pdf	1217098 bcf4c7b7a55190f3168a8ea18183e7372994f2d8	no	11
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Information:					
7	Non Patent Literature	Croll_2004.pdf	2647465 622951c3c1068cf513951f9878c58568c890ab4d	no	15
Warnings:					
Information:					
8	Non Patent Literature	DeVriese_2001.pdf	472800 315d7ee33b1ea94e70b3c2865ed739fbffbb6c83	no	8
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9	Non Patent Literature	Eremina_2001.pdf	4088162 24fe6cadc852d68ab47d929eac59854d2373a3ed	no	10
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10	Non Patent Literature	Eriksson_and_Alitalo.pdf	2081053 0a8d888a86fb2a6feed4a4cde01201eaff906a68	no	17
Warnings:					
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11	Non Patent Literature	Ferrara.pdf	3622466 ee47a2652aae7189cbb40aa871921d0fb894747e	no	30
Warnings:					
Information:					
12	Non Patent Literature	Ferrara_1999.pdf	437001 1062f3fbd144b35ad7169cb169448a7aac2ab9e3	no	6
Warnings:					
Information:					
13	Non Patent Literature	Flyvbjerg_2002.pdf	137211 f16c80ee6a4d6123ed86ac9a82a2dcc1fb09e27f	no	5
Warnings:					
Information:					
14	Non Patent Literature	Holash_1999.pdf	1253369 3b1a7a106cecffd3ece27755b634ac865ebaa231	no	6
Warnings:					
Information:					
15	Non Patent Literature	Korobelnik_2014.pdf	475993 a018a34e0e817b45dc88156395903a71df424106	no	7
Warnings:					
Information:					

16	Non Patent Literature	Mitchell_2013.pdf	2555569 aa12fff21717962d60a0f87ccaae3586ef3f5c62	no	13
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Information:					
17	Non Patent Literature	Noguera-Troise_2006.pdf	4146971 41fa22c0bc3a6e37d0b1d0b3f90f2c1a5413daa9	no	6
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18	Non Patent Literature	Rudge_2007.pdf	2813823 c03cb1fcf59ebe11e95d7e2d7094407b49be5260	no	8
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19	Non Patent Literature	Schmidt-Erfurth_2014.pdf	647240 3dc7c75d15c920d816a7c2c519e2d6ff72485267	no	9
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Information:					
20	Non Patent Literature	Semeraro_2013.pdf	239051 9c73e3927d9432e267858cf261f42789ddf8ca9a	no	12
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21	Non Patent Literature	Tannock_2013.pdf	264317 7699deca5f6f643b791e1b7f84a8b8705e664be1	no	9
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22	Non Patent Literature	Thurston_2002.pdf	430961 cd5fea4cd11d72c0b3f1cd4c029ea57ddd818398	no	6
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23	Non Patent Literature	Xia_Blood_2013.pdf	2098399 07488287a13cc7c26c9adf9542d1998a9a491e29	no	8
Warnings:					
Information:					
		Total Files Size (in bytes):	34912031		

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-008CIPCON5
	Confirmation No.	8135
	First Named Inventor	George D. Yancopoulos
	Application Number	16/397,267
	Filing Date	April 29, 2019
	Group Art Unit	
Address to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Examiner Name	
	Title: "*Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders*"	

Sir:

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-008CIPCON5.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 31 March 2020

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

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/397,267	04/29/2019	George D. YANCOPOULOS	REGN-008CIPCON5	8135

96387 7590 05/12/2020

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
05/12/2020	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

Mylan Exhibit 1017
Mylan v. Regeneron, IPR2022-01226
Page 796

Samsung Bioepis Exhibit 1017, pg. 796

Office Action Summary	Application No. 16/397,267	Applicant(s) YANCOPOULOS, George D.	
	Examiner JON M LOCKARD	Art Unit 1647	AIA (FITF) Status No

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

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 07 January 2020.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

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

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 21-63 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 21-63 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 29 April 2019 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

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

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

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

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

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

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

Mylan Exhibit 1017
Mylan v. Regeneron, IPR2022-01226
Page 797

Samsung Bioepis Exhibit 1017, pg. 797

Notice of Pre-AIA or AIA Status

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

DETAILED ACTION

Status of Application, Amendments, and/or Claims

2. The Preliminary Amendment filed on 07 January 2020 has been entered in full. Claims 1-20 have been cancelled, and claims 21-63 have been added. Therefore, claims 21-63 are pending and the subject of this Office Action.

Information Disclosure Statement

3. The information disclosure statements (IDS) filed 19 June 2019, 18 September 2019, 27 January 2020, 21 February 2020 and 31 March 2020 have been considered by the examiner.

Double Patenting

4. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van*

Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

5. A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

6. The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit http://www.uspto.gov/forms/. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

7. Claims 21-63 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-26 of U.S. Patent No. 9,254,338. Although the conflicting claims are not identical, as they recite different dosing schedules, they are not patentably distinct from each other because claims 1-26 of the '338 patent are drawn to a method for treating an angiogenic eye disorder, including age-related macular degeneration, diabetic retinopathy, choroidal neovascularization, vascular leak, and/or retinal edema, comprising administering a fusion polypeptide having the amino acid sequence set forth in SEQ ID NO:2, which comprises an immunoglobin-like (Ig) domain 2 of a first VEGF receptor (VEGFR1) and Ig domain 3 of a second VEGF receptor (VEGFR2) and a multimerizing component, which is what aflibercept

comprises. While the '338 patent does not disclose the dosing schedules set forth in the instant claims, it is routine experimentation to optimize dosages and dosage schedules. The courts have determined that:

> "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller,* 220 F.2d 454, 454, 105 USPQ 223,235, (CCPA 1955).

Therefore, the claims are overlapping in scope.

8. Claims 21-63 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-12 of U.S. Patent No. 9,669,069. Although the conflicting claims are not identical, as they recite different dosing schedules, they are not patentably distinct from each other because claims 1-12 of the '069 patent are drawn to a method for treating an angiogenic eye disorder, including age-related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization, comprising administering a fusion polypeptide having the amino acid sequence set forth in SEQ ID NO:2, which comprises an immunoglobin-like (Ig) domain 2 of a first VEGF receptor (VEGFR1) and Ig domain 3 of a second VEGF receptor (VEGFR2) and a multimerizing component, which is what aflibercept comprises. While the '069 patent does not disclose the dosing schedules set forth in the instant claims, it is routine experimentation to optimize dosages and dosage schedules. The courts have determined that:

> "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller,* 220 F.2d 454, 454, 105 USPQ 223,235, (CCPA 1955).

Therefore, the claims are overlapping in scope.

9. Claims 21-63 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-12 of U.S. Patent No. 10,130,681. Although the conflicting claims are not identical, as they recite different dosing schedules, they are not patentably distinct from each other because claims 1-12 of the '681 patent are drawn to a method for treating an angiogenic eye disorder, including age-related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization, comprising administering a fusion polypeptide having the amino acid sequence set forth in SEQ ID NO:2, which comprises an immunoglobin-like (Ig) domain 2 of a first VEGF receptor (VEGFR1) and Ig domain 3 of a second VEGF receptor (VEGFR2) and a multimerizing component, which is what aflibercept comprises. While the '681 patent does not disclose the dosing schedules set forth in the instant claims, it is routine experimentation to optimize dosages and dosage schedules. The courts have determined that:

> "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller,* 220 F.2d 454, 454, 105 USPQ 223,235, (CCPA 1955).

Therefore, the claims are overlapping in scope.

10. Claims 21-63 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 32-42 of co-pending U.S. Application No. 16/159,282 (reference application). Although the conflicting claims are not identical, as they recite different dosing schedules, they are not patentably distinct from each other because claims 32-42 of the '282 Application are drawn to a method for treating an angiogenic eye disorder, including age-related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization, comprising

administering a VEGF antagonist, wherein the VEGF comprises an immunoglobin-like (Ig) domain 2 of Flt1 and Ig domain 3 of Flk1 and a multimerizing component, or aflibercept. While the '282 Application does not disclose the dosing schedules set forth in the instant claims, it is routine experimentation to optimize dosages and dosage schedules. The courts have determined that:

> "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller,* 220 F.2d 454, 454, 105 USPQ 223,235, (CCPA 1955).

Therefore, the claims are overlapping in scope.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented, although a Notice of Allowability has been mailed (01 April 2020).

Summary

11. No claim is allowed.

Advisory Information

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

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joanne Hama, can be reached on (571) 272-2911. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

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

/JON M LOCKARD/
Examiner, Art Unit 1647
May 6, 2020

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/397,267	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		05/06/2020	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.	05/06/2020	JML
STN (MEDLINE, SCISEARCH, EMBASE, BIOSIS): See attached search history.	05/06/2020	JML
PALM: Inventor search.	05/06/2020	JML

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

Mylan Exhibit 1017
Mylan v. Regeneron, IPR2022-01226
Page 804

Samsung Bioepis Exhibit 1017, pg. 804

Inventor Information for 16/397267

/J.L./

Inventor Name	City	State/Country
YANCOPOULOS, GEORGE D.	YORKTOWN HEIGHTS	NEW YORK

Appln Info | Contents | Petition Info | Atty/Agent Info | Continuity Data | Foreign Data | **Inventors** | Applicants | Address | Fees | Post Info | Pre Gr

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Back to PALM | ASSIGNMENT | OASIS | Home page

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Page 805

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EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	7990	(flt1 or vegfr1 or (vegf adj r1)) same ((flk1 or kdr or vegfr2 or (vegf adj r2)) or (Flt4 vegfr3 or (vegf adj r3)))	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2020/05/06 21:30
L2	2035	l1 and ((chimer$ or fusion) same vegf)	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2020/05/06 21:31
L3	856	l1 same ((chimer$ or fusion) same vegf)	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2020/05/06 21:31
L4	7799	(flt1 or vegfr1 or (vegf adj r1)) with ((flk1 or kdr or vegfr2 or (vegf adj r2)) or (Flt4 vegfr3 or (vegf adj r3)))	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2020/05/06 21:31
L5	442	l4 with ((chimer$ or fusion) with vegf)	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2020/05/06 21:31
L6	2467	(l4 l5) and ((eye or ocular or retina$ or macular) with disorder)	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2020/05/06 21:32
L7	382	(l3 l5) and ((eye or ocular or retina$ or macular) with disorder)	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2020/05/06 21:32
L8	27	(l3 l5) same ((eye or ocular or retina$ or macular) with disorder)	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2020/05/06 21:32
L9	482	yancopoulos-g$.in.	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2020/05/06 21:32
L10	49	l7 and l9	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2020/05/06 21:32

L11	17	l10 and (eye ocular macular).clm.	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2020/05/06 21:33

5/6/2020 9:34:04 PM
C:\Users\jlockard\Documents\EAST\Workspaces\16397267.wsp

Receipt date: 02/21/2020

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	George D. Yancopoulos
				Art Unit	
				Examiner Name	
Sheet	1	of	4	Attorney Docket Number	REGN-008CIPCON5

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

Receipt date: 02/21/2020

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	George D. Yancopoulos
				Art Unit	
				Examiner Name	
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	6	Updated Information from ClinicalTrials.gov archive History of Changes for Study: NCT01012973 "Vascular Endothelial Growth Factor (VEGF) Trap-Eye: Investigation of Efficacy and Safety in Central Retinal Vein Occlusion (CRVO)(GALILEO) 11 pages, Latest version submitted October 27, 2014 on ClinicalTrials.gov (NCT01012973_02202012_27427.1)	
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Examiner Signature	/JON M LOCKARD/	Date Considered	05/06/2020

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	2	16/159,282 – Third Party Submissions dated May 31, 2019	
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Examiner Signature	/JON M LOCKARD/	Date Considered	05/06/2020

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(FILE 'HOME' ENTERED AT 21:36:28 ON 06 MAY 2020)

FILE 'MEDLINE, SCISEARCH, EMBASE, BIOSIS' ENTERED AT 21:37:04 ON 06 MAY 2020

L1 4654 S (FLT1 OR VEGFR1 OR (VEGF (W) R1)) (S) ((FLK1 OR KDR OR VEGFR2
L2 23 S L1 (S) ((CHIMER? OR FUSION) (S) VEGF)
L3 12 DUP REM L2 (11 DUPLICATES REMOVED)
L4 1766 S VEGF (W) TRAP
L5 12838 S AFLIBERCEPT
L6 8030 S (L3 OR L4 OR L5) (P) (EYE OR OCULAR OR RETINA? OR MACULAR)
L7 6108 S (L3 OR L4 OR L5) (S) (EYE OR OCULAR OR RETINA? OR MACULAR)
L8 123 S L7 AND 2MG
L9 91 DUP REM L8 (32 DUPLICATES REMOVED)
E YANCOPOULOS G/AU
L10 2258 S E3 OR E4 OR E5 OR E8 OR E9
L11 0 S L9 AND L10
L12 52 S L7 AND L10
L13 24 DUP REM L12 (28 DUPLICATES REMOVED)

Receipt date: 09/18/2019

INFORMATION DISCLOSURE STATEMENT BY APPLICANT					
				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	Yancopoulos, George D.
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	1	of	3	Attorney Docket Number	REGN-008CIPCON5

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	1	**2008/0220004**	2008-09-11	Wiegand et al.	
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				Examiner Name	Jon McClelland Lockard
Sheet	3	of	3	Attorney Docket Number	REGN-008CIPCON5

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SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
16/397,267	04/29/2019 RULE	424	1647	REGN-008CIPCON5

APPLICANTS
REGENERON PHARMACEUTICALS, INC., Tarrytown, NY

INVENTORS
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**** CONTINUING DATA ***************************
This application is a CON of 16/159,282 10/12/2018
which is a CON of 15/471,506 03/28/2017 PAT 10130681
which is a CON of 14/972,560 12/17/2015 PAT 9669069
which is a CON of 13/940,370 07/12/2013 PAT 9254338
which is a CIP of PCT/US2012/020855 01/11/2012
which claims benefit of 61/432,245 01/13/2011
and claims benefit of 61/434,836 01/21/2011
and claims benefit of 61/561,957 11/21/2011

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****
05/08/2019

Foreign Priority claimed ❑ Yes ☑ No 35 USC 119(a-d) conditions met ❑ Yes ❑ No Verified and Acknowledged /JON MCCLELLAND LOCKARD/ Examiner's Signature	❑ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		NY	1	29	4

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UNITED STATES

TITLE
USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

FILING FEE RECEIVED	FEES: Authority has been given in Paper	❑ All Fees
4940	No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	❑ 1.16 Fees (Filing) ❑ 1.17 Fees (Processing Ext. of time) ❑ 1.18 Fees (Issue) ❑ Other _____ ❑ Credit

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
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				First Named Inventor	YANCOPOULOS, GEORGE D.
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	2	7303748	2007-12-04	Wiegand	
	3	7306799	2007-12-11	Wiegand	
	4	7396664	2008-07-08	Daly et al.	
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	7	10130681	2018-11-20	Yancopoulos	

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	2	WO 2000/75319	2000-12-14	Regeneron Pharmaceuitcals, Inc.		
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	4	WO 2008/063932	2008-05-29	Genentech, Inc.		
	5	JP 2010-509369	2010-03-25	Genentech, Inc.	See WO 2008/063932 for English Equivalent	
	6	WO 2012/097019	2012-07-19	Regeneron Pharmaceuticals, Inc.		

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				Examiner Name	Jon McClelland Lockard
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	1	7070959	2006-07-04	Papadopoulos	
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	1	2019/0388539	2019-12-26	Dix et al.	
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Sheet	2	of	2	Attorney Docket Number	REGN-008CIPCON5

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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
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Examiner Signature	/JON M LOCKARD/	Date Considered	05/06/2020

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	George D. Yancopoulos
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	1	of	2	Attorney Docket Number	REGN-008CIPCON5

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				
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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
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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
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	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)	
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	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)	
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	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)	
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Bayer



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Investor News

Final Phase 2 Results Presented at Retina Society Meeting

VEGF Trap-Eye: New Data Confirm Successes in the Treatment of Age-related Macular Degeneration

Statistically significant reduction in the size of the affected area of the retina demonstrated

Leverkusen, Germany, September 28, 2008 – VEGF Trap-Eye can achieve durable improvements in visual acuity and in biologic measurement parameters in the formation of new blood vessels in the treatment of age-related macular degeneration (AMG). This was shown in the final evaluation of a Phase 2 study presented at the annual meeting of the Retina Society in Scottsdale, Arizona. These parameters include retinal thickness and active choroidal neovascularization lesion size (the damaged part of the retina). Bayer HealthCare and Regeneron Pharmaceuticals, Inc (Nasdaq:REGN) are developing VEGF Trap-Eye together. The treatment successes continued for up to a year.

The study showed that VEGF Trap-Eye was also associated with a reduction in the size of the choroidal neovascular membrane (CNV), the active lesion that is the underlying cause of vision loss in patients with wet AMD. Patients receiving monthly doses of VEGF Trap-Eye of either 2.0 or 0.5 milligrams (mg) for 12 weeks followed by PRN dosing achieved mean improvements in visual acuity versus baseline of 9.0 letters ($p<0.0001$ versus baseline) and 5.4 letters ($p<0.085$ versus baseline), respectively. Patients in the 2.0 mg monthly cohort also achieved a statistically significant 1.75 mm^2 reduction in total lesion size. A reduction in total lesion size was not seen in the cohort initially dosed with 0.5 mg monthly.

"Progression of the active CNV lesion and resulting vision impairment are an inevitable consequence of untreated wet AMD. The reduction in total active CNV lesion size achieved with VEGF Trap-Eye treatment in this Phase 2 clinical study could potentially translate into clinically meaningful outcomes in the larger, controlled Phase 3 studies that

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are underway," stated Jason Slakter, M.D., head of the independent reading center for the study and a Clinical Professor of Ophthalmology, New York University School of Medicine, New York.

In this double-masked Phase 2 trial, participants were initially treated with either monthly or quarterly fixed dosing for 12 weeks and then continued to receive treatment for another 40 weeks on a PRN (as needed) dosing schedule. Patients receiving fixed monthly doses of VEGF Trap-Eye of either 2.0 or 0.5 milligrams (mg) for 12 weeks (i.e. 4 fixed doses) followed by PRN dosing achieved mean improvements in visual acuity versus baseline of 9.0 letters ($p<0.0001$ versus baseline) and 5.4 letters ($p<0.085$ versus baseline), respectively, at the end of one year. The proportion of patients with vision of 20/40 or better (part of the legal minimum medical requirement for an unrestricted driver's license in the U.S.) increased from 23 percent at baseline to 45 percent at week 52 in patients initially treated with 2.0 mg monthly and from 16 percent at baseline to 47 percent at week 52 in patients initially treated with 0.5 mg monthly. During the week 12 to week 52 PRN dosing period, patients initially dosed on a 2.0 mg monthly schedule received, on average, only 1.6 additional injections and those initially dosed on a 0.5 mg monthly schedule received, on average, 2.5 injections.

Patients receiving monthly doses of VEGF Trap-Eye of either 2.0 or 0.5 mg for 12 weeks followed by PRN dosing also achieved mean decreases in retinal thickness versus baseline of 143 microns ($p<0.0001$ versus baseline) and 125 microns ($p<0.0001$ versus baseline) at week 52, respectively.

While PRN dosing following a fixed quarterly dosing regimen (with dosing at baseline and week 12) also yielded improvements in visual acuity and retinal thickness versus baseline at week 52, the results generally were not as robust as those obtained with initial monthly treatment.

"Anti-VEGF therapy has dramatically changed the treatment paradigm for wet AMD, and improvement in visual acuity is now feasible in most patients. The biggest challenge we have is that with our current drugs, the majority of patients need frequent injections into their eye to maintain their visual acuity gains," stated David M. Brown, M.D., a study investigator and a retinal specialist at The Methodist Hospital in Houston."These study results reinforce our interest in further exploring whether continued administration of

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VEGF Trap-Eye on an as-needed basis after an initial period of fixed dosing can maintain a durability of effect over time in controlled Phase 3 clinical studies."

VEGF Trap-Eye was generally well tolerated and there were no drug-related serious adverse events. There was one reported case of eye inflammation (culture-negative endophthalmitis/uveitis) in the study eye, which was deemed not to be drug-related. The most common adverse events were those typically associated with intravitreal injections.

About the Phase 3 Program in Wet AMD

Regeneron and Bayer HealthCare initiated a Phase 3 global development program for VEGF Trap-Eye in wet AMD in August 2007. In two Phase 3 trials, VIEW 1 and VIEW 2 (VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet age related macular degeneration), the companies are evaluating VEGF Trap-Eye dosed 0.5 mg every 4 weeks, 2 mg every 4 weeks, or 2 mg every 8 weeks (following three monthly doses) in direct comparison with ranibizumab (Lucentis®, a registered trademark of Genentech, Inc.) administered 0.5 mg every four weeks according to its U.S. label during the first year of the studies. PRN dosing will be evaluated during the second year of each study. The VIEW 1 study (http://www.regeneron.com/vegftrap_eye.html) is currently enrolling patients in the United States and Canada and the VIEW 2 study (www.view2study.com) is currently enrolling patients in Europe, Asia Pacific, Japan and Latin America. The companies are collaborating on the global development of VEGF Trap-Eye for the treatment of wet AMD, diabetic eye diseases, and other eye diseases and disorders. Bayer HealthCare will market VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of VEGF Trap-Eye. Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States.

About VEGF Trap-Eye

Vascular Endothelial Growth Factor (VEGF) is a naturally occurring protein in the body whose normal role is to trigger formation of new blood vessels (angiogenesis) to support the growth of the body's tissues and organs. It has also been associated with the abnormal growth and fragility of new blood vessels in the eye, which lead to the development of wet AMD. The VEGF Trap-Eye is a fully human, soluble VEGF receptor fusion protein that binds all forms of VEGF-A along with the related placental growth factor (PlGF). VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. Blockade of VEGF, which can prevent abnormal blood vessel formation and vascular leak, has proven beneficial in the treatment of wet AMD.

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About Wet AMD

Age-related Macular Degeneration (AMD) is a leading cause of acquired blindness. Macular degeneration is diagnosed as either dry (nonexudative) or wet (exudative). In wet AMD, new blood vessels grow beneath the retina and leak blood and fluid. This leakage causes disruption and dysfunction of the retina creating blind spots in central vision, and it can account for blindness in wet AMD patients. Wet AMD is the leading cause of blindness for people over the age of 65 in the U.S. and Europe.

About Bayer HealthCare

The Bayer Group is a global enterprise with core competencies in the fields of health care, nutrition and high-tech materials. Bayer HealthCare, a subsidiary of Bayer AG, is one of the world's leading, innovative companies in the healthcare and medical products industry and is based in Leverkusen, Germany. The company combines the global activities of the Animal Health, Consumer Care, Diabetes Care and Pharmaceuticals divisions. The pharmaceuticals business operates under the name Bayer Schering Pharma. Bayer HealthCare's aim is to discover and manufacture products that will improve human and animal health worldwide. Find more information at www.bayerhealthcare.com.

Bayer Schering Pharma is a worldwide leading specialty pharmaceutical company. Its research and business activities are focused on the following areas: Diagnostic Imaging, General Medicine, Specialty Medicine and Women's Healthcare. With innovative products, Bayer Schering Pharma aims for leading positions in specialized markets worldwide. Using new ideas, Bayer Schering Pharma aims to make a contribution to medical progress and strives to improve the quality of life. Find more information at www.bayerscheringpharma.de.

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Forward-Looking Statements
This release may contain forward-looking statements based on current assumptions and forecasts made by Bayer Group or subgroup management. Various known and unknown risks, uncertainties and other factors could lead to material differences between the actual future results, financial situation, development or performance of the company and the estimates given here. These factors include those discussed in Bayer's public reports which are available on the Bayer website at www.bayer.com. The company assumes no liability whatsoever to update these forward-looking statements or to conform them to future events or developments.

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REGENERON

Positive Interim Phase 2 Data Reported for VEGF Trap-Eye in Age-Related Macular Degeneration

March 27, 2007

Positive Interim Phase 2 Data Reported for VEGF Trap-Eye in Age-Related Macular DegenerationTARRYTOWN, N.Y. & LEVERKUSEN, Germany, Mar 27, 2007 (BUSINESS WIRE) -- Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) and Bayer HealthCare AG (NYSE: BAY) today announced positive preliminary data from a pre-planned interim analysis of a Phase 2 randomized study of their VEGF Trap-Eye in patients with the neovascular form of age-related macular degeneration (wet AMD). The VEGF Trap-Eye met its primary endpoint of a statistically significant reduction in retinal thickness after 12 weeks compared with baseline (all groups combined, decrease of 135 microns, $p < 0.0001$). Mean change from baseline in visual acuity, a key secondary endpoint of the study, also demonstrated statistically significant improvement (all groups combined, increase of 5.9 letters, $p < 0.0001$). Moreover, patients in the dose groups that received only a single dose, on average, demonstrated a decrease in excess retinal thickness ($p < 0.0001$) and an increase in visual acuity ($p = 0.012$) at 12 weeks. There were no drug-related serious adverse events, and treatment with the VEGF Trap-Eye was generally well-tolerated. The most common adverse events were those typically associated with intravitreal injections. Detailed data from this interim analysis will be presented at an upcoming scientific conference.

"These data support our efforts to develop the VEGF Trap as a potent blocker of VEGF in various diseases," said George D. Yancopoulos, M.D., Ph.D., President of Regeneron Research Laboratories. "Importantly, the VEGF Trap-Eye may offer the potential to improve vision in patients with wet AMD with dosing less frequently than every four weeks. Our Phase 3 program is being designed to test this possibility and further evaluate the safety and efficacy of various doses and dosing intervals of the VEGF Trap-Eye."

"We are very pleased with the outcome of this interim analysis and the findings support the potential of the VEGF Trap-Eye to improve the lives of patients suffering from wet AMD, which accounts for 90% of AMD related blindness," said Kemal Malik, M.D., member of the Bayer HealthCare Executive Committee, responsible for Global Development. "These results encourage us in our plans to foster next steps in development and to further study the VEGF Trap-Eye in additional eye diseases."

Based on these results, Regeneron and Bayer HealthCare AG plan to initiate the VEGF Trap-Eye Phase 3 program in the second half of 2007. The companies are collaborating on the global development of the VEGF Trap-Eye for the treatment of wet AMD, diabetic eye diseases, and other eye diseases and disorders. Bayer HealthCare AG and Regeneron will jointly commercialize the VEGF Trap-Eye outside the United States, and Regeneron maintains exclusive rights in the United States.

The Phase 2 study is a 12-week, multi-center trial involving 150 patients who are randomized to 5 groups and treated with the VEGF Trap-Eye in one eye. Two groups received either 0.5 or 2.0 mg of VEGF Trap-Eye administered every four weeks, and three groups received a single dose of 0.5, 2.0, or 4.0 mg of VEGF Trap-Eye. Patients are monitored for safety, retinal thickness, and visual acuity over 12 weeks. Retinal thickness is determined by optical coherence tomography (OCT) scans read at an independent reading center. Visual acuity is defined as the total number of letters read correctly on the Early Diabetic Retinopathy Study (ETDRS) chart. Maintenance of vision is defined as losing fewer than 3 lines (equivalent to 15 letters) on the ETDRS chart.

The interim analysis was conducted on the first 78 patients who completed 12 weeks of study. As summarized above, overall, patients had a statistically significant improvement in retinal thickness and visual acuity. All but one patient maintained or improved vision at 12 weeks. Although the improvement in visual acuity was numerically larger in patients receiving injections every 4 weeks, there were no statistically significant differences across the five dose groups in either retinal thickness or visual acuity at 12 weeks.

About the VEGF Trap-Eye

Vascular endothelial growth factor (VEGF) is a naturally occurring protein in the body whose normal role is to trigger formation of new blood vessels (angiogenesis) to support the growth of the body's tissues and organs. It has also been associated with the abnormal growth and fragility of new blood vessels in the eye, which lead to the development of wet AMD. The VEGF Trap-Eye is a fully human, soluble VEGF receptor fusion protein that binds all forms of VEGF-A along with the related placental growth factor (PlGF). The VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. Blockade of VEGF, which can prevent abnormal blood vessel formation and vascular leak, has proven beneficial in the treatment of wed AMD.

About AMD

Age-related macular degeneration (AMD) is a leading cause of acquired blindness. Patients with condition can experience a loss of vision due to the development of abnormal, fragile blood vessels in the back of the eye. A particular type of AMD, called wet AMD, accounts for approximately 90% of AMD-related blindness. Wet AMD is the leading cause of blindness for people over the age of 65 in the U.S. and Europe.

Macular degeneration is diagnosed as either dry (nonexudative) or wet (exudative). In wet AMD, new blood vessels grow beneath the retina and leak blood and fluid. This leakage causes disruption and dysfunction of the retina creating blind spots in central vision and can account for blindness in wet AMD patients.

About Regeneron Pharmaceuticals

Regeneron is a biopharmaceutical company that discovers, develops, and intends to commercialize therapeutic medicines for the treatment of serious medical conditions. Regeneron has therapeutic candidates for the potential treatment of cancer, eye diseases, and inflammatory diseases and has

preclinical programs in other diseases and disorders.

About Bayer HealthCare

Bayer HealthCare, a subsidiary of Bayer AG, is one of the world's leading, innovative companies in the healthcare and medical products industry and is based in Leverkusen, Germany. The company combines the global activities of the Animal Health, Consumer Care, Diabetes Care and Pharmaceuticals divisions. The pharmaceuticals business operates under the name Bayer Schering Pharma AG. Bayer HealthCare's aim is to discover and manufacture products that will improve human and animal health worldwide.

This news release discusses historical information and includes forward-looking statements about Regeneron and its products, programs, finances, and business, all of which involve a number of risks and uncertainties, such as risks associated with preclinical and clinical development of our drug candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict our ability to continue to develop or commercialize our drug candidates, competing drugs that are superior to our product candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any collaboration agreement, including our agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or to terminate without any product success, risks associated with third party intellectual property, and other material risks. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission (SEC), including its Form 10-K for the year ended December 31, 2006. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise unless required by law.

This news release contains forward-looking statements based on current assumptions and forecasts made by Bayer Group management. Various known and unknown risks, uncertainties and other factors could lead to material differences between the actual future results, financial situation, development or performance of the company and the estimates given here. These factors include those discussed in our public reports filed with the Frankfurt Stock Exchange and with the U.S. Securities and Exchange Commission (including our Form 20-F). The company assumes no liability whatsoever to update these forward-looking statements or to conform them to future events or developments.

Additional information about Regeneron and recent news releases are available on Regeneron's worldwide web site at www.regeneron.com

SOURCE: Regeneron Pharmaceuticals, Inc.

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REGENERON

VEGF Trap-Eye Phase 2 Wet AMD Results Reported at ARVO Annual Meeting

May 9, 2007
VEGF Trap-Eye Phase 2 Wet AMD Results Reported at ARVO Annual MeetingAverage Vision Gain of More Than 10 Letters at 12 Weeks in Highest Monthly Dose Group
Single Dose Results in Average Vision Gain at 12 Weeks
Phase 3 wet AMD Program to Begin in the Third Quarter of 2007

FORT LAUDERDALE, Fla.--(BUSINESS WIRE)--May 9, 2007--Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) today announced positive results from several studies evaluating the VEGF Trap-Eye in the neovascular form of age-related macular degeneration (wet AMD) and diabetic macular edema (DME). These findings were presented this week at the annual meeting of the Association for Research in Vision and Ophthalmology (ARVO). The data reported at the meeting from a pre-planned interim analysis of a Phase 2 randomized study of the VEGF Trap-Eye in patients with wet AMD and a Phase 1 DME trial are available on the Regeneron website (www.regeneron.com on the Events page, under the Investor Relations heading).

"We are very encouraged by the preliminary observation in the Phase 2 wet AMD trial that the most intense dosing regimen studied, 2 milligrams delivered by intravitreal injection every 4 weeks, resulted in an average gain of more than 10 letters after 12 weeks of treatment," said George D. Yancopoulos, M.D., Ph.D., President of Regeneron Research Laboratories. "Perhaps equally important is that after receiving only a single dose of the VEGF Trap-Eye, patients on average had an improvement in the number of letters read both 8 and 12 weeks after treatment. Although significantly more clinical testing is required, the VEGF Trap-Eye may offer the potential to improve vision in patients with wet AMD with a regular dosing regimen that is less frequent than monthly."

Regeneron and Bayer HealthCare AG plan to initiate the VEGF Trap-Eye Phase 3 program in wet AMD in the third quarter of 2007. In the first Phase 3 trial, the companies currently plan to evaluate the VEGF Trap-Eye using 4 and 8 week dosing intervals in direct comparison with ranibizumab (Lucentis®, a registered trademark of Genentech, Inc.) administered every 4 weeks according to its label. The companies are collaborating on the global development of the VEGF Trap-Eye for the treatment of wet AMD, diabetic eye diseases, and other eye diseases and disorders. Bayer HealthCare AG and Regeneron will jointly commercialize the VEGF Trap-Eye outside the United States, and Regeneron maintains exclusive rights in the United States.

In the Phase 2 wet AMD trial, data were presented from a pre-planned interim analysis of the first 78 patients who completed 12 weeks of the study. The randomized, multi-center trial involves 150 patients who were randomized to 5 groups and treated with the VEGF Trap-Eye in one eye. Two groups received either 0.5 or 2.0 milligrams (mg) of VEGF Trap-Eye administered every 4 weeks, and three groups received a single dose of 0.5, 2.0, or 4.0 mg of VEGF Trap-Eye. Patients were monitored for safety, retinal thickness, and visual acuity over 12 weeks. The VEGF Trap-Eye met its primary endpoint of a statistically significant reduction in retinal thickness after 12 weeks compared with baseline (all groups combined, decrease of 135 microns, p less than 0.0001). Mean change in visual acuity, a key secondary endpoint of the study, also demonstrated a statistically significant improvement (all groups combined, increase of 5.9 letters, p less than 0.0001). There were no drug-related serious adverse events, and treatment with the VEGF Trap-Eye was generally well-tolerated. The most common adverse events were those typically associated with intravitreal injections. Interim data for all dose groups were presented at the ARVO meeting. The Phase 2 wet AMD study is now fully enrolled and results for all patients will be presented at a future scientific meeting.

Encouraging results were also presented from a Phase 1 study of VEGF Trap-Eye in DME. In this open-label safety study, the VEGF Trap-Eye was administered as a single 4.0 mg intravitreal injection to 5 patients with longstanding diabetes and multiple prior treatments for DME. The single injection resulted in a marked decrease in mean central retinal thickness and mean macular volume throughout the 6 week observation period. The VEGF Trap-Eye was generally well tolerated, and there were no drug-related serious adverse events. Adverse events were mostly related to the injection procedure.

About the VEGF Trap-Eye

Vascular endothelial growth factor (VEGF) is a naturally occurring protein in the body whose normal role is to trigger formation of new blood vessels (angiogenesis) to support the growth of the body's tissues and organs. It has also been associated with the abnormal growth and fragility of new blood vessels in the eye, which lead to the development of wet AMD. The VEGF Trap-Eye is a fully human, soluble VEGF receptor fusion protein that binds all forms of VEGF-A along with the related placental growth factor (PlGF). The VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. Blockade of VEGF, which can prevent abnormal blood vessel formation and vascular leak, has proven beneficial in the treatment of wet AMD. Blocking VEGF has been shown to be effective in patients with wet AMD; and a VEGF inhibitor, ranibizumab, has been approved for treatment of patients with this condition.

About AMD

Age-related macular degeneration (AMD) is a leading cause of acquired blindness. Patients with this condition can experience a loss of vision due to the development of abnormal, fragile blood vessels in the back of the eye. A particular type of AMD, called wet AMD, accounts for approximately 90% of AMD-related blindness. Wet AMD is the leading cause of blindness for people over the age of 65 in the U.S. and Europe.

Macular degeneration is diagnosed as either dry (nonexudative) or wet (exudative). In wet AMD, new blood vessels grow beneath the retina and leak blood and fluid. This leakage causes disruption and dysfunction of the retina creating blind spots in central vision, and it can account for blindness in wet AMD patients.

About DME

Diabetic Macular Edema (DME) is the most prevalent cause of moderate vision loss in patients with diabetes. DME is a common complication of Diabetic Retinopathy (DR), a disease affecting the blood vessels of the retina. A leading cause of blindness in younger adults (under 50), DME occurs when fluid leaks into the center of the macula, the light-sensitive part of the retina responsible for sharp, direct vision. Fluid in the macula can cause severe vision loss or blindness.

Approximately 500,000 Americans currently suffer from DME, with 75,000 new cases arising each year. According to the American Diabetes Association, more than 18 million Americans currently suffer from diabetes and many other people are at risk for developing diabetes. With the incidence of diabetes steadily climbing, it is projected that up to 10 percent of all patients with diabetes will develop DME during their lifetime.

About Regeneron Pharmaceuticals

Regeneron is a biopharmaceutical company that discovers, develops, and intends to commercialize therapeutic medicines for the treatment of serious medical conditions. Regeneron has therapeutic candidates for the potential treatment of cancer, eye diseases, and inflammatory diseases and has preclinical programs in other diseases and disorders.

This news release discusses historical information and includes forward-looking statements about Regeneron and its products, programs, finances, and business, all of which involve a number of risks and uncertainties, such as risks associated with preclinical and clinical development of our drug candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict our ability to continue to develop or commercialize our drug candidates, competing drugs that are superior to our product candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any collaboration agreement, including our agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or to terminate without any product success, risks associated with third party intellectual property, and other material risks. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission (SEC), including its Form 10-Q for the quarter ended March 31, 2007. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise unless required by law.

Additional information about Regeneron and recent news releases are available on Regeneron's worldwide web site at www.regeneron.com

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SOURCE: Regeneron Pharmaceuticals, Inc.

REGENERON

Regeneron Reports Second Quarter Financial and Operating Results

August 1, 2007
Regeneron Reports Second Quarter Financial and Operating ResultsTARRYTOWN, N.Y.--(BUSINESS WIRE)--Aug. 1, 2007--Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) today announced financial and operating results for the second quarter of 2007. The Company reported a net loss of $26.8 million, or $0.41 per share (basic and diluted), for the second quarter of 2007 compared with a net loss of $23.6 million, or $0.41 per share (basic and diluted), for the second quarter of 2006. The Company reported a net loss of $56.7 million, or $0.86 per share (basic and diluted), for the six months ended June 30, 2007 compared with a net loss of $44.0 million, or $0.77 per share (basic and diluted), for the same period in 2006.

At June 30, 2007, cash, restricted cash, and marketable securities totaled $512.3 million compared with $522.9 million at December 31, 2006. In the first quarter of 2007, the Company entered into non-exclusive license agreements with AstraZeneca UK Limited and Astellas Pharma Inc. with respect to the Company's VelocImmune® technology for generating human monoclonal antibody product candidates, as described below. In connection with these agreements, AstraZeneca and Astellas each made an up-front payment to the Company of $20.0 million in February and April 2007, respectively.

The Company's $200.0 million of convertible notes, which bear interest at 5.5% per annum, mature in October 2008.

Current Business Highlights

Regeneron is currently focused on three clinical development programs: rilonacept (IL-1 Trap) in various inflammatory indications, aflibercept (VEGF Trap) in oncology in collaboration with the sanofi-aventis Group, and the VEGF Trap-Eye in eye diseases in collaboration with Bayer HealthCare AG. The Company also is developing its pipeline of preclinical antibody candidates discovered utilizing its VelocImmune technology.

Key planned milestones for the third quarter of 2007 include:

-- FDA acceptance of the BLA submission for rilonacept for CAPS and establishment of target completion date for FDA review of BLA.

-- Reporting of results of the Phase 2 trial for the VEGF Trap-Eye in wet AMD.

-- Initiation of the Phase 3 program for the VEGF Trap-Eye in wet AMD and receipt of a milestone payment from Bayer HealthCare upon initiation of the Phase 3 program.

-- Initiation of the Phase 3 program for the VEGF Trap in oncology in combination with standard chemotherapy regimens.

-- Full enrollment of 200 patients in the Phase 2 single-agent VEGF Trap study in advanced ovarian cancer, which was achieved in July.

-- Reporting of results of an exploratory proof-of-concept trial of rilonacept in gout and initiation of a safety and efficacy trial in gout.

-- Completion of preparation for advancing our first human monoclonal antibody product candidate into clinical trials in the fourth quarter.

Rilonacept - Inflammatory Diseases

The Company announced in June that it had completed the rolling submission of a Biologics License Application (BLA) to the U.S. Food and Drug Administration (FDA) for rilonacept (IL-1 Trap) for the long-term treatment of Cryopyrin-Associated Periodic Syndromes (CAPS). CAPS is a spectrum of rare inherited inflammatory conditions, including Familial Cold Autoinflammatory Syndrome and Muckle-Wells Syndrome.

The FDA has previously granted Orphan Drug status and Fast Track designation to rilonacept for the treatment of CAPS. Rilonacept has also received Orphan Drug designation in the European Union for the treatment of CAPS.

Regeneron also is evaluating the potential use of rilonacept in other indications in which IL-1 may play a role. The Company is completing an exploratory proof of concept study of rilonacept in ten patients with chronic gout, and a safety and efficacy trial of rilonacept in patients with gout is planned to begin this quarter. The Company also expects to initiate an exploratory proof of concept study of rilonacept in another indication in the fourth quarter.

VEGF Trap - Eye Diseases

The VEGF Trap-Eye is a specially purified and formulated form of the VEGF Trap for use in intraocular applications. Regeneron and Bayer HealthCare plan to initiate the VEGF Trap-Eye Phase 3 program in the neovascular form of age-related macular degeneration (wet AMD) this quarter. The first Phase 3 trial will compare the VEGF Trap-Eye and Genentech, Inc.'s Lucentis® (ranibizumab), an anti-angiogenic agent approved for use in wet AMD. This Phase 3 trial will evaluate dosing intervals of four and eight weeks for the VEGF Trap-Eye, compared with ranibizumab dosing according to its label every four weeks. In May 2007, the companies announced positive preliminary results for a pre-planned interim analysis of the Phase 2 trial of the VEGF Trap-Eye in wet AMD. The companies expect to report full results of the Phase 2 trial in the third quarter. Regeneron and Bayer HealthCare plan to initiate a second Phase 3 trial in wet AMD around the end of 2007.

The companies are collaborating on the global development of the VEGF Trap-Eye for the treatment of wet AMD, diabetic eye diseases, and other eye diseases and disorders. Bayer HealthCare and Regeneron will jointly commercialize the VEGF Trap-Eye outside the United States, and Regeneron maintains exclusive rights in the United States. The development program in eye disease is expected to total over $250 million over the next several years, with the Company and Bayer HealthCare sharing the costs.

VEGF Trap - Oncology

Regeneron and sanofi-aventis are preparing to initiate a large Phase 3 program that will combine the VEGF Trap with standard chemotherapy regimens in five different advanced solid tumors: colorectal, non-small cell lung, prostate, pancreas and gastric cancer. The companies expect the first Phase 3 trial to begin in the current quarter. The development program in oncology is expected to total over $400 million over the next several years, which will be funded by sanofi-aventis.

In June 2007, at the annual meeting of the American Society of Clinical Oncology (ASCO), Regeneron and sanofi-aventis announced interim results of two Phase 2 single-agent studies of the VEGF Trap in patients with advanced ovarian cancer (AOC) and non-small cell lung adenocarcinoma (NSCLA). The companies are also conducting a Phase 2 trial of the VEGF Trap in AOC patients with symptomatic malignant ascites (SMA).

The AOC study, selected for an oral presentation at ASCO, was an interim analysis of a Phase 2 randomized, double-blind, multi-center trial investigating two doses of the VEGF Trap used as a single agent in patients with recurrent platinum-resistant epithelial ovarian cancer. While the study remains blinded with regards to dose, the combined preliminary results of the two dose levels for 162 of a planned 200 patients demonstrated anti-tumor activity, as evidenced by an 8.0 percent partial response rate and 77 percent achievement of stable disease at 4 weeks in heavily pre-treated patients who had failed multiple other treatments. The VEGF Trap has been well tolerated, and the most common adverse events have been the typical class effect of anti-angiogenic agents. Of the 23 patients in the AOC study with evaluable baseline ascites, 7 patients (30 percent) experienced complete disappearance of the ascites, and 13 patients (57 percent) experienced no increase in ascites during treatment. The AOC study is ongoing and is now fully enrolled.

The second study, presented as a poster at ASCO, is a Phase 2 single-arm study conducted in patients with platinum-resistant and erlotinib-resistant adenocarcinoma of the lung. In this study, the preliminary results demonstrated activity in this heavily pre-treated patient base, as evidenced by a 3.7 percent partial response rate and 63 percent of patients achieving stable disease. The VEGF Trap has been well-tolerated in this trial as well. This study is ongoing and is now fully enrolled.

Sanofi-aventis has indicated that a first registration submission to a regulatory agency for the VEGF Trap is possible as early as 2008.

The companies have also initiated their first trial of the VEGF Trap in Japan, a Phase 1 safety and tolerability study in combination with S-1 in patients with advanced solid malignancies. In addition, currently underway or scheduled to begin are more than 12 studies to be conducted in conjunction with the National Cancer Institute (NCI) Cancer Therapy Evaluation Program (CTEP) evaluating the VEGF Trap as a single agent or in combination with chemotherapy regimens in a variety of cancer indications.

Monoclonal Antibodies

VelocImmune, Regeneron's novel technology for producing fully human monoclonal antibodies, is part of the Company's suite of proprietary, inter-related technology platforms that are designed to provide Regeneron with its next generation of therapeutic candidates. Regeneron plans to move its first new antibody product candidate into clinical trials in the fourth quarter of 2007, with plans to advance at least two antibody product candidates into human clinical trials each year going forward.

In 2007, Regeneron entered into non-exclusive license agreements with AstraZeneca and Astellas that will allow those companies to utilize VelocImmune technology in their internal research programs to discover human monoclonal antibody product candidates. Each of those companies made a $20.0 million up-front, non-refundable payment and will make up to five additional annual payments of $20.0 million, subject to the ability to terminate the agreement after making the first three additional payments. Upon commercialization of any antibody products discovered utilizing VelocImmune, the licensees will pay to Regeneron a mid-single-digit royalty on product sales.

Financial Results

Revenue

Regeneron's total revenue increased to $22.2 million in the second quarter of 2007 from $19.3 million in the same quarter of 2006 and to $38.0 million for the first six months of 2007 from $37.5 million for the same period of 2006. Contract research and development revenue in the first half of 2007 and 2006 principally related to the Company's VEGF Trap collaboration with sanofi-aventis in cancer indications. Contract manufacturing revenue in 2006 related to Regeneron's long-term manufacturing agreement with Merck & Co., Inc., which expired in October 2006. Technology licensing revenue in the first half of 2007 related to the Company's license agreements with AstraZeneca and Astellas.

Regeneron recognized contract research and development revenue of $13.5 million in the second quarter of 2007 and $25.3 million for the first six months of 2007 related to the Company's collaboration with sanofi-aventis, compared with $14.8 million and $28.7 million, respectively, for the same periods of 2006. Contract research and development revenue from the sanofi-aventis collaboration consisted of reimbursement of VEGF Trap development expenses plus recognition of amounts related to $105.0 million of previously received and deferred up-front, non-refundable payments. Reimbursement of expenses decreased to $11.3 million in the second quarter of 2007 from $11.8 million in the comparable quarter of 2006, and to $20.8 million in the first six months of 2007 from $22.6 million in the same period of 2006, principally because costs related to the Company's manufacture of VEGF Trap clinical supplies were lower in 2007. With respect to the up-front payments from sanofi-aventis, $2.2 million was recognized in the second quarter of 2007 compared to $3.0 million in the same quarter of 2006, and $4.5 million was recognized in the first six months of 2007 compared to $6.1 million in the same period of 2006.

Sanofi-aventis also incurs VEGF Trap development expenses directly and these expenses are increasing because of the growing number of clinical trials sanofi-aventis is overseeing in the VEGF Trap oncology program. During the term of the collaboration, sanofi-aventis pays 100% of agreed-upon VEGF Trap development expenses incurred by both companies. Following commercialization of a VEGF Trap product by the collaboration, Regeneron, from its 50% share of VEGF Trap profits, will reimburse sanofi-aventis for 50% of the VEGF Trap development expenses previously paid by sanofi-aventis.

In connection with the Company's license agreements with AstraZeneca and Astellas, both of the $20.0 million non-refundable, up-front payments received in February and April 2007, respectively, were deferred and are being recognized as revenue ratably over approximately the first year of each agreement. In the second quarter and for the first six months of 2007, the Company recognized $6.3 million and $8.4 million, respectively, of technology licensing revenue related to these agreements.

Bayer HealthCare Collaboration

In October 2006, the Company entered into a collaboration with Bayer HealthCare for the development and commercialization of the VEGF Trap-Eye outside the United States, and received a $75.0 million up-front, non-refundable payment. In 2007, agreed upon VEGF Trap-Eye development expenses incurred by both companies under a global development plan will be shared as follows: Up to the first $50.0 million will be shared equally; Regeneron is solely responsible for the next $40.0 million; over $90.0 million will be shared equally. Through June 30, 2007, reimbursements from Bayer HealthCare of our VEGF Trap-Eye development expenses totaled $10.6 million. All payments received or receivable from Bayer HealthCare through June 30, 2007, totaling $85.6 million, have been fully deferred and included in deferred revenue for financial statement purposes.

Expenses

Total operating expenses for the second quarter of 2007 were $52.8 million, 21 percent higher than the same period in 2006, and $102.2 million for the first six months of 2007, 23 percent higher than the same period in 2006. Operating expenses included non-cash compensation expense related to employee stock option awards (Stock Option Expense) of $6.9 million in the second quarter of 2007 and $13.5 million for the first six months of 2007, compared with $4.6 million and $8.5 million, respectively, for the same periods of 2006. The increase in total Stock Option Expense in 2007 was primarily due to the higher fair market value of the Company's Common Stock on the date of annual employee option grants made by the Company in December 2006 in comparison to the fair market value of the Company's Common Stock on the dates of annual employee option grants made in recent prior years.

Research and development (R&D) expenses increased to $43.9 million in the second quarter of 2007 from $34.4 million in the comparable quarter of 2006, and to $85.1 million in the first six months of 2007 from $66.5 million in the same period of 2006. In addition to the impact of Stock Option Expense, as described above, in the first half of 2007, the Company incurred higher R&D costs related to additional headcount and additional clinical manufacturing capacity, and higher costs related to preclinical development of new antibody candidates and clinical development of the VEGF Trap-Eye and rilonacept. These were partly offset by lower development expenses for the VEGF Trap cancer program.

General and administrative (G&A) expenses increased to $8.9 million in the second quarter of 2007 from $6.3 million in the comparable quarter of 2006, and to $17.1 million in the first six months of 2007 from $12.2 million in the same period of 2006. In addition to the impact of Stock Option Expense, as described above, in the first half of 2007, the Company incurred higher G&A costs related to additional headcount and higher fees for various professional services.

About Regeneron Pharmaceuticals

Regeneron is a biopharmaceutical company that discovers, develops, and intends to commercialize therapeutic medicines for the treatment of serious medical conditions. Regeneron has therapeutic candidates in clinical trials for the potential treatment of cancer, eye diseases, and inflammatory diseases, and has preclinical programs in other diseases and disorders.

This news release discusses historical information and includes forward-looking statements about Regeneron and its products, programs, finances, and business, all of which involve a number of risks and uncertainties, such as risks associated with preclinical and clinical development of our drug candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict our ability to continue to develop or commercialize our drug candidates, competing drugs that are superior to our product candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any collaboration agreement, including our agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or to terminate without any product success, risks associated with third party intellectual property, and other material risks. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission (SEC), including its Form 10-K for the year ended December 31, 2006 and Form 10-Q for the quarter ended March 31, 2007. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise unless required by law.

REGENERON PHARMACEUTICALS, INC.
CONDENSED BALANCE SHEETS (Unaudited)
(In thousands)

	June 30, 2007	December 31, 2006	
ASSETS			
Cash, restricted cash, and marketable securities	$512,282	$522,859	
Receivables	20,478	7,493	
Property, plant, and equipment, net	47,647	49,353	
Other assets	17,451	5,385	
Total assets	$597,858	$585,090	
LIABILITIES AND STOCKHOLDERS' EQUITY			
Accounts payable and accrued expenses	$34,905	$21,471	
Deferred revenue	183,617	146,995	
Notes payable	200,000	200,000	
Stockholders' equity	179,336	216,624	
Total liabilities and stockholders' equity	$597,858	$585,090	

REGENERON PHARMACEUTICALS, INC.
CONDENSED STATEMENTS OF OPERATIONS (Unaudited)
(In thousands, except per share data)

	For the three months ended June 30,		For the six months ended June 30,	
	2007	2006	2007	2006
Revenues				
Contract research and development	$15,917	$14,991	$29,562	$29,578
Contract manufacturing	4,267		7,899	
Technology licensing	6,278		8,421	
	22,195	19,258	37,983	37,477
Expenses				
Research and development	43,864	34,398	85,099	66,482
Contract manufacturing	2,810		4,662	
General and administrative	8,935	6,299	17,137	12,245
	52,799	43,507	102,236	83,389
Loss from operations	(30,604)	(24,249)	(64,253)	(45,912)

Other income (expense)				
Investment income	6,841	3,684	13,584	7,165
Interest expense	(3,011)	(3,011)	(6,022)	(6,022)
	3,830	673	7,562	1,143
Net loss before cumulative effect of a change in accounting principle	(26,774)	(23,576)	(56,691)	(44,769)
Cumulative effect of adopting Statement of Financial Accounting Standards No. 123R ("SFAS 123R")				813
Net loss	($26,774)	($23,576)	($56,691)	($43,956)
Net loss per share amounts, basic and diluted:				
Net loss before cumulative effect of a change in accounting principle	($0.41)	($0.41)	($0.86)	($0.79)
Cumulative effect of adopting SFAS 123R				0.02
Net loss	($0.41)	($0.41)	($0.86)	($0.77)
Weighted average shares outstanding, basic and diluted	65,950	56,915	65,757	56,821

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SOURCE: Regeneron Pharmaceuticals, Inc.

REGENERON

Regeneron and Bayer HealthCare Initiate Phase 3 Global Development Program For VEGF Trap-Eye In Wet Age-Related Macular Degeneration (AMD)

August 2, 2007

Regeneron and Bayer HealthCare Initiate Phase 3 Global Development Program For VEGF Trap-Eye In Wet Age-Related Macular Degeneration (AMD)TARRYTOWN, N.Y. & LEVERKUSEN, Germany--(BUSINESS WIRE)--Aug. 2, 2007--Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) and Bayer HealthCare AG (NYSE:BAY) announced today that the companies have initiated a Phase 3 study of the VEGF Trap-Eye in the neovascular form of age-related macular degeneration (wet AMD). The study will be a non-inferiority comparison of the VEGF Trap-Eye and ranibizumab (Lucentis®, a registered trademark of Genentech, Inc.), an anti-angiogenic agent approved for use in wet AMD. The study will be conducted pursuant to a Special Protocol Assessment from the U.S. Food and Drug Administration (FDA). This trial, known as VIEW 1 (VEGF Trap: Investigation of Efficacy and safety in Wet age-related macular degeneration), is the first study in the companies' Phase 3 global development program in wet AMD, which is planned to be carried out in the U.S., Europe, and other parts of the world.

"Age-related macular degeneration continues to be one of the leading causes of blindness in adults, and new therapies are essential to providing optimal patient care," stated Jeffrey Heier, M.D., a clinical ophthalmologist at Ophthalmic Consultants of Boston and chair of the steering committee for the trial. "The results of early phase studies of VEGF Trap-Eye suggest it has the potential to be a highly efficacious treatment with less frequent administration. If these results are confirmed in Phase 3 trials, it would be important for both patients and physicians and would be a significant advance in the treatment of these patients."

"The initiation of this Phase 3 trial represents a major milestone in the development of the VEGF Trap-Eye to treat wet AMD," said Avner Ingerman, M.D., vice president and ophthalmology team leader for Regeneron. "While this trial enables us to continue in our effort to improve the lives of patients suffering from wet AMD, it also signals the beginning of a larger, more global development program investigating the potential of VEGF Trap-Eye for the treatment of diabetic eye diseases and other eye diseases and disorders."

The randomized, double-masked Phase 3 study is expected to enroll approximately 1,200 patients in more than 200 centers throughout the United States and Canada. The study will evaluate the safety and efficacy of the VEGF Trap-Eye at doses of 0.5 milligrams (mg) and 2.0 mg administered at four-week dosing intervals and 2.0 mg at an eight-week dosing interval, compared to 0.5 mg of ranibizumab administered every four weeks, consistent with its labeled dosing schedule.

The primary endpoint of the study is the proportion of patients treated with the VEGF Trap-Eye who maintain or improve vision at the end of one year, compared to ranibizumab patients. Visual acuity is defined as the total number of letters read correctly on the Early Treatment Diabetic Retinopathy Study (ETDRS) chart. Maintenance of vision is defined as losing fewer than three lines (equivalent to 15 letters) on the ETDRS chart. After the first year of treatment, patients will continue to be treated and followed for another year.

In an analysis of interim data from the ongoing Phase 2 trial in wet AMD, where patients were treated with the VEGF Trap-Eye either monthly or quarterly, combined data for all patients demonstrated a statistically significant reduction in retinal thickness and improvement in visual acuity after 12 weeks, compared to baseline. There were no drug-related serious adverse events, and treatment with the VEGF Trap-Eye was generally well-tolerated. The most common adverse events were those typically associated with intravitreal injections. The interim results of this Phase 2 trial were presented at the annual meeting of the Association for Research in Vision and Ophthalmology (ARVO) this past May. The companies expect to report final primary endpoint results of the trial at a scientific meeting later this quarter.

Regeneron and Bayer HealthCare are collaborating on the global development of the VEGF Trap-Eye for the treatment of wet AMD, diabetic eye diseases, and other eye diseases and disorders. Bayer HealthCare will market the VEGF Trap-Eye outside the United States, where the parties will share equally in profits from any future sales of the VEGF Trap-Eye. Regeneron maintains exclusive rights to the VEGF Trap-Eye in the United States.

About the VEGF Trap-Eye

Vascular endothelial growth factor (VEGF) is a naturally occurring protein in the body whose normal role is to trigger formation of new blood vessels (angiogenesis) to support the growth of the body's tissues and organs. It has also been associated with the abnormal growth and fragility of new blood vessels in the eye, which lead to the development of wet AMD. The VEGF Trap-Eye is a fully human, soluble VEGF receptor fusion protein that binds all forms of VEGF-A along with the related placental growth factor (PlGF). The VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. Blockade of VEGF, which can prevent abnormal blood vessel formation and vascular leak, has proven beneficial in the treatment of wet AMD. Blocking VEGF has been shown to be effective in patients with wet AMD; and a VEGF inhibitor, ranibizumab, has been approved for treatment of patients with this condition.

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About Bayer HealthCare

The Bayer Group is a global enterprise with core competencies in the fields of health care, nutrition and high-tech materials. Bayer HealthCare, a subsidiary of Bayer AG, is one of the world's leading, innovative companies in the healthcare and medical products industry and is based in Leverkusen, Germany. The company combines the global activities of the Animal Health, Consumer Care, Diabetes Care and Pharmaceuticals divisions. The pharmaceuticals business operates under the name Bayer Schering Pharma AG. Bayer HealthCare's aim is to discover and manufacture products that will improve human and animal health worldwide. Find more information at www.bayerhealthcare.com.

Forward Looking Statement - Regeneron

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Forward-Looking Statements - Bayer HealthCare

This news release contains forward-looking statements based on current assumptions and forecasts made by Bayer Group management. Various known and unknown risks, uncertainties and other factors could lead to material differences between the actual future results, financial situation, development or performance of the company and the estimates given here. These factors include those discussed in our annual and interim reports to the Frankfurt Stock Exchange and in our reports filed with the U.S. Securities and Exchange Commission (including our Form 20-F). The company assumes no liability whatsoever to update these forward-looking statements or to conform them to future events or developments.

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SOURCE: Regeneron Pharmaceuticals, Inc.

REGENERON

Regeneron Announces Positive Primary Endpoint Results from a Phase 2 Study of VEGF Trap-Eye in Age-related Macular Degeneration

October 1, 2007

Regeneron Announces Positive Primary Endpoint Results from a Phase 2 Study of VEGF Trap-Eye in Age-related Macular DegenerationTARRYTOWN, N.Y.--(BUSINESS WIRE)--Oct. 1, 2007--Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) and development partner, Bayer HealthCare AG (NYSE:BAY) of Leverkusen, Germany, today announced positive results from the full analysis of the primary 12-week endpoint of a Phase 2 study evaluating the VEGF Trap-Eye in the neovascular form of age-related macular degeneration (wet AMD). The VEGF Trap-Eye met the primary study endpoint of a statistically significant reduction in retinal thickness, a measure of disease activity, after 12 weeks of treatment compared with baseline (all five dose groups combined, mean decrease of 119 microns, p<0.0001). The mean change from baseline in visual acuity, a key secondary endpoint of the study, also demonstrated statistically significant improvement (all groups combined, increase of 5.7 letters, p<0.0001). Preliminary analyses at 16 weeks showed that the VEGF Trap-Eye, dosed monthly, achieved a mean gain in visual acuity of 9.3 to 10 letters (for the 0.5 and 2 mg dose groups, respectively). In additional exploratory analyses, the VEGF Trap-Eye, dosed monthly, reduced the proportion of patients with vision of 20/200 or worse (a generally accepted definition for legal blindness) from 14.3 percent at baseline to 1.6 percent at week 16; the proportion of patients with vision of 20/40 or better (part of the legal minimum requirement for an unrestricted driver's license in the U.S.) was likewise increased from 19.0 percent at baseline to 49.2 percent at 16 weeks. These findings were presented at the Retina Society Conference in Boston, MA. The data reported at the meeting are available on the Regeneron website (www.regeneron.com on the Events Page, under the Investor Relations heading).

In this double-masked, prospective, randomized, multi-center Phase 2 trial, 157 patients were randomized to five groups and treated with the VEGF Trap-Eye in one eye. Two groups received monthly doses of 0.5 or 2.0 milligrams (mg) of VEGF Trap-Eye and three groups received quarterly doses of 0.5, 2.0, or 4.0 mg of VEGF Trap-Eye (at baseline and week 12). Patients were monitored for safety, retinal thickness, and visual acuity. All five dose groups showed an improvement in retinal thickness and an increase in mean letters read versus baseline at all time points through week 12. There were no drug-related ocular or systemic serious adverse events (SAE) reported. Treatment with the VEGF Trap-Eye was generally well tolerated. The most common adverse events were those typically associated with intravitreal injections.

Preliminary week 16 results showed that retinal thickness for all groups combined continued to improve with a mean decrease of 159 microns versus baseline (p<0.0001). The mean change from baseline in visual acuity also continued to improve (all groups combined, increase of 6.6 letters versus baseline, p<0.0001). Patients receiving monthly doses of the VEGF Trap-Eye, either 0.5 or 2 mg, achieved mean decreases in retinal thickness of 160 and 183 microns, respectively, and mean improvements in visual acuity of 9.3 and 10 letters, respectively, at week 16. While quarterly dosing improved retinal thickness and visual acuity versus baseline at 12 and 16 weeks, the effect was not as robust as with monthly dosing. A single 2-mg dose maintained similar effect on visual acuity as 2 mg dosed monthly out to eight weeks (5.8 vs. 6.2 letters gained at 8 weeks, respectively). The table below summarizes preliminary 16-week results for patients in each dosing arm of the study.

"We are particularly encouraged by the decrease, following monthly treatment, in the proportion of patients with vision at the legally blind level of 20/200 or worse, as well as the proportion of patients whose vision improved to 20/40 or better," said George D. Yancopoulos, M.D., Ph.D., President of Regeneron Research Laboratories. "Our large Phase 3 program will help us determine the full impact of the VEGF Trap-Eye on visual acuity in these patient populations with significant unmet clinical needs."

"These results reaffirm the decision to study both the 0.5 mg and 2 mg monthly doses in the Phase 3 program," stated Jeffrey Heier, M.D., a clinical ophthalmologist at Ophthalmic Consultants of Boston, a primary investigator in the Phase 2 study, and chair of the steering committee for the Phase 3 VIEW 1 trial. "The quarterly dosing arms seemed to sustain their effect on visual acuity out to eight weeks, providing the rationale for exploring an eight-week dosing schedule in the Phase 3 program. Further improvement in visual acuity and dosing convenience continue to represent major unmet medical needs in the treatment of wet AMD."

VEGF Trap Dose:	0.5 mg (n=32)	2 mg q4wk (n=31)	0.5 mg q4wk (n=32)	2 mg q12wk (n=31)	4 mg q12wk (n=31)	q12wk
Retinal thickness (mean decrease in microns) at 16 wks	160	183	135	107	210	
Visual acuity (mean letters gained) at 16 wks	9.3	10.0	5.6	4.3	3.9	

% of patients who gained 15 or more letters at 16 wks	25%	39%	22%	19%	10%
% of patients with 20/40 vision or better:					
-At Baseline	16%	23%	22%	10%	16%
-At Week 16	44%	55%	31%	36%	32%
% of patients with 20/200 vision or less:					
-At Baseline	19%	10%	9%	7%	19%
-At Week 16	3%	0%	13%	7%	13%

About the Phase 3 Program in Wet AMD

Regeneron and Bayer HealthCare AG initiated a Phase 3 global development program for the VEGF Trap-Eye in wet AMD in August of this year. In the first Phase 3 trial, the companies will evaluate the VEGF Trap-Eye using four- and eight-week dosing intervals in direct comparison with ranibizumab (Lucentis®, a registered trademark of Genentech, Inc.) administered every four weeks according to its label. The Phase 3 wet AMD study is currently being enrolled. The companies are collaborating on the global development of the VEGF Trap-Eye for the treatment of wet AMD, diabetic eye diseases, and other eye diseases and disorders. Bayer HealthCare will market the VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of the VEGF Trap-Eye. Regeneron maintains exclusive rights in the United States.

About the VEGF Trap-Eye

Vascular endothelial growth factor (VEGF) is a naturally occurring protein in the body whose normal role is to trigger formation of new blood vessels (angiogenesis) to support the growth of the body's tissues and organs. It has also been associated with the abnormal growth and fragility of new blood vessels in the eye, which lead to the development of wet AMD. The VEGF Trap-Eye is a fully human, soluble VEGF receptor fusion protein that binds all forms of VEGF-A along with the related placental growth factor (PlGF). The VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. Blockade of VEGF, which can prevent abnormal blood vessel formation and vascular leak, has proven beneficial in the treatment of wet AMD and a VEGF inhibitor, ranibizumab, has been approved for treatment of patients with this condition.

About Wet AMD

Age-related macular degeneration (AMD) is a leading cause of acquired blindness. Macular degeneration is diagnosed as either dry (nonexudative) or wet (exudative). In wet AMD, new blood vessels grow beneath the retina and leak blood and fluid. This leakage causes disruption and dysfunction of the retina creating blind spots in central vision, and it can account for blindness in wet AMD patients. Wet AMD is the leading cause of blindness for people over the age of 65 in the U.S. and Europe.

About Regeneron Pharmaceuticals

Regeneron is a biopharmaceutical company that discovers, develops, and intends to commercialize therapeutic medicines for the treatment of serious medical conditions. Regeneron has therapeutic candidates for the potential treatment of cancer, eye diseases, and inflammatory diseases and has preclinical programs in other diseases and disorders. Additional information about Regeneron and recent news releases are available on Regeneron's worldwide web site at www.regeneron.com

Forward Looking Statement - Regeneron

This news release discusses historical information and includes forward-looking statements about Regeneron and its products, programs, finances, and business, all of which involve a number of risks and uncertainties, such as risks associated with preclinical and clinical development of our drug candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict our ability to continue to develop or commercialize our drug candidates, competing drugs that are superior to our product candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any collaboration agreement, including our agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or to terminate without any product success, risks associated with third party intellectual property, and other material risks. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission (SEC), including its Form 10-Q for the quarter ended June 30, 2007. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise unless required by law.

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REGENERON

Regeneron Reports Fourth Quarter and Full Year 2007 Financial and Operating Results

February 27, 2008
Regeneron Reports Fourth Quarter and Full Year 2007 Financial and Operating ResultsTARRYTOWN, N.Y.--(BUSINESS WIRE)--Feb. 27, 2008--Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) today announced financial and operating results for the fourth quarter and full year 2007. The Company reported a net loss of $13.1 million, or $0.19 per share (basic and diluted), for the fourth quarter of 2007 compared with a net loss of $31.0 million, or $0.51 per share (basic and diluted), for the fourth quarter of 2006. The Company reported a net loss of $105.6 million, or $1.59 per share (basic and diluted), for the year ended December 31, 2007 compared with a net loss of $102.3 million, or $1.77 per share (basic and diluted), for the same period in 2006. In the fourth quarter of 2007, in connection with the Company's VEGF Trap-Eye collaboration with Bayer HealthCare, the Company recognized a cumulative catch-up of $35.9 million of contract research and development revenue and $10.6 million of additional research and development expense, as described below.

At December 31, 2007, cash, restricted cash, and marketable securities totaled $846.3 million compared with $522.9 million at December 31, 2006. In November 2007, the Company and the sanofi-aventis Group entered into a global, strategic collaboration to discover, develop, and commercialize fully human monoclonal antibodies and sanofi-aventis made an $85.0 million up-front payment to Regeneron. In addition, in December 2007, sanofi-aventis purchased 12 million newly issued shares of Regeneron Common Stock at $26.00 per share for proceeds to the Company of $312.0 million.

The Company's $200.0 million of convertible notes, which bear interest at 5.5 percent per annum, mature in October 2008.

Current Business Highlights

Regeneron has three late-stage clinical development programs: ARCALYST (rilonacept; also known as IL-1 Trap) in Cryopyrin-Associated Periodic Syndromes (CAPS), aflibercept (the VEGF Trap) in oncology in collaboration with the sanofi-aventis Group, and the VEGF Trap-Eye in eye diseases in collaboration with Bayer HealthCare. Regeneron has also initiated a Phase 2 trial of ARCALYST for the prevention of gout.

In addition, Regeneron has commenced a Phase 1 trial of its first fully human monoclonal antibody candidate, REGN88, an antibody targeting the interleukin-6 receptor (IL-6R) in rheumatoid arthritis, as part of its antibody collaboration with sanofi-aventis. The Company is developing a pipeline of preclinical antibody candidates utilizing its VelocImmune® technology.

Regeneron achieved the following milestones in the fourth quarter of 2007:

- Entered into a global, strategic collaboration agreement with sanofi-aventis to discover, develop, and commercialize fully human monoclonal antibodies.
- Reported extended safety results from a Phase 3 trial of ARCALYST(TM) in patients with CAPS at the American College of Rheumatology (ACR) Annual Meeting in November 2007.
- Initiated a Phase 2 safety and efficacy trial of ARCALYST(TM) in the prevention of gout flares.
- Reported positive results from the extension phase of the Phase 2 trial of the VEGF Trap-Eye in age-related macular degeneration (wet AMD).
- Initiation by sanofi-aventis of the third and fourth Phase 3 oncology trials for aflibercept in combination with standard chemotherapy regimens.
- Initiated a Phase 1 clinical trial of REGN88 in rheumatoid arthritis.

ARCALYST (rilonacept; also known as IL-1 Trap) - Inflammatory Diseases

The Company announced in November 2007 that the action date for the FDA's priority review of the Biologics License Application (BLA) for ARCALYST for the long-term treatment of CAPS was set for February 29, 2008. CAPS is a group of rare inherited inflammatory conditions, including Familial Cold Auto-inflammatory Syndrome and Muckle-Wells Syndrome. The FDA previously granted Orphan Drug status and Fast Track designation to ARCALYST for the treatment of CAPS. ARCALYST has also received Orphan Drug designation in the European Union for the treatment of CAPS.

In the fourth quarter, Regeneron initiated a Phase 2 safety and efficacy trial of ARCALYST in the prevention of gout flares induced by the initiation of uric acid-lowering drug therapy used to control the disease. The Company had previously reported positive results from an exploratory proof-of-concept study of ARCALYST in ten patients with chronic active gout. In those patients, treatment with ARCALYST demonstrated a statistically significant reduction in patient pain scores in the single-blind, placebo-controlled study. Mean patients' pain scores, the key symptom measure in persistent gout, were reduced 41 percent (p=0.025) during the first two weeks of active treatment and reduced 56 percent (pless than0.004) after six weeks of active treatment. In this study, in which safety was the primary endpoint measure, treatment with ARCALYST was generally well-tolerated.

Regeneron is evaluating the potential use of ARCALYST in other indications in which interleukin-1 (IL-1) may play a role.

Aflibercept (VEGF Trap) - Oncology

In December 2007, Regeneron and sanofi-aventis announced the initiation of the third and fourth Phase 3 trials in oncology that combine aflibercept with standard chemotherapy regimens. One trial is evaluating aflibercept in combination with folinic acid, 5-FU, and irinotecan in patients with 2nd line metastatic colorectal cancer. The other trial is evaluating aflibercept in combination with gemcitabine in patients with 1st line metastatic pancreatic cancer. In the first two Phase 3 trials initiated by the collaboration, aflibercept is being evaluated in combination with docetaxel/prednisone in patients with 1st line metastatic androgen independent prostate cancer and in combination with docetaxel in patients with 2nd line metastatic non-small cell lung cancer. All four trials are studying the current standard of chemotherapy care for the cancer being studied with or without aflibercept. In addition, currently underway are 10 studies being conducted in conjunction with the National Cancer Institute (NCI) Cancer Therapy Evaluation Program (CTEP) evaluating aflibercept as a single agent or in combination with chemotherapy regimens in a variety of cancer indications.

VEGF Trap-Eye - Eye Diseases

The VEGF Trap-Eye is a specially purified and formulated form of the VEGF Trap for use in intraocular applications. Regeneron and Bayer HealthCare initiated a Phase 3 global development program of the VEGF Trap-Eye in wet AMD in the third quarter of 2007. The first trial, known as VIEW 1 (VEGF Trap: Investigation of Efficacy and Safety in Wet age-related macular degeneration), is comparing the VEGF Trap-Eye and Genentech, Inc.'s Lucentis® (ranibizumab), an anti-angiogenic agent approved for use in wet AMD. The trial is evaluating dosing intervals of four and eight weeks for the VEGF Trap-Eye, compared with ranibizumab dosed every four weeks according to its label. Regeneron and Bayer HealthCare plan to initiate a second Phase 3 trial in wet AMD in the first half of 2008. This second trial will be conducted primarily in the European Union and other parts of the world outside the U.S.

In the fourth quarter of 2007, the companies announced positive results of the Phase 2 trial of the VEGF Trap-Eye in wet AMD. The VEGF Trap-Eye met the primary study endpoint of a statistically significant reduction in retinal thickness, a measure of disease activity, after 12 weeks of treatment compared with baseline (all five dose groups combined, mean decrease of 119 microns, pless than0.0001). In additional exploratory analyses, the VEGF Trap-Eye, dosed monthly, demonstrated improvements in visual acuity. The VEGF Trap-Eye reduced the proportion of patients with vision of 20/200 or worse (a generally accepted definition for legal blindness), from 14.3 percent at baseline to 1.6 percent at week 16. In a separate analysis, the proportion of patients with vision of 20/40 or better (part of the legal minimum requirement for an unrestricted driver's license in the U.S.) was increased from 19.0 percent at baseline to 49.2 percent at 16 weeks.

Regeneron and Bayer HealthCare are collaborating on the global development of the VEGF Trap-Eye for the treatment of wet AMD, diabetic eye diseases, and other eye diseases and disorders. Bayer HealthCare will market the VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of the VEGF Trap-Eye. Regeneron maintains exclusive rights to the VEGF Trap-Eye in the United States.

Monoclonal Antibodies

In the fourth quarter of 2007, Regeneron and sanofi-aventis entered into a global, strategic collaboration agreement to discover, develop, and commercialize fully human monoclonal antibodies. The first therapeutic antibody to enter clinical development under the collaboration, REGN88, is an antibody to the Interleukin-6 receptor (IL-6R), which has started clinical trials in rheumatoid arthritis. The second is expected to be an antibody to Delta-like ligand-4 (Dll4), which is currently slated to start clinical development in mid-2008. Regeneron plans to advance two antibody product candidates into clinical development in 2008 and an additional two to three antibody product candidates each year thereafter beginning in 2009.

The collaboration is governed by a Discovery and Preclinical Development Agreement and a License and Collaboration Agreement. As part of the discovery agreement, sanofi-aventis made an $85.0 million up-front payment to Regeneron. In addition, sanofi-aventis agreed to fund up to $475.0 million of research over the next five years to identify and validate potential drug discovery targets and to develop fully human monoclonal antibodies against these targets. Sanofi-aventis has an option to extend the discovery agreement for up to an additional three years.

Sanofi-aventis has the exclusive option under the license agreement to co-develop antibodies arising from Regeneron's discovery efforts. Sanofi-aventis will fund the drug candidate development costs up-front and Regeneron will reimburse sanofi-aventis for half of the development costs from its share of future antibody profits from the collaboration.

For any product successfully developed as part of the collaboration, sanofi-aventis will take the lead in commercialization activities and Regeneron has worldwide co-promotion rights. In the United States, profits and losses from sales of collaboration antibodies will be shared equally. Outside the United States, profits will be split on a pre-determined sliding scale based on aggregate sales of collaboration antibodies with Regeneron's' share ranging from 35 percent to 45 percent. Regeneron is responsible for 45 percent of losses outside the United States. In addition, Regeneron is entitled to receive up to a total of $250.0 million of sales milestone payments when the collaboration antibodies achieve certain aggregate annual ex-U.S. sales levels, starting at $1.0 billion.

In December 2007, sanofi-aventis also increased its ownership of Regeneron's outstanding Common Stock from approximately 4 percent to approximately 19 percent by purchasing 12 million newly issued shares of Regeneron Common Stock at $26.00 per share for proceeds to the Company of $312.0 million.

Earlier in 2007, Regeneron entered into non-exclusive license agreements with AstraZeneca and Astellas that will allow those companies to utilize VelocImmune technology in their internal research programs to discover human monoclonal antibody product candidates. Each of those companies made a $20.0 million up-front, non-refundable payment and will make up to five additional annual payments of $20.0 million, subject to the ability to

terminate the agreement after making the first three additional payments. Upon commercialization of any antibody products discovered utilizing VelocImmune, the licensees will pay to Regeneron a mid-single-digit royalty on product sales.

Financial Results

Revenue

Regeneron's total revenue increased to $64.7 million in the fourth quarter of 2007 from $10.3 million in the same quarter of 2006 and to $125.0 million for the full year 2007 from $63.4 million for the same period of 2006. Contract research and development revenue in the first three quarters of 2007 and the full-year 2006 principally related to the Company's aflibercept collaboration with sanofi-aventis in cancer indications. In the fourth quarter of 2007, the Company also recognized contract research and development revenue from the Company's VEGF Trap-Eye collaboration with Bayer HealthCare and its new collaboration with sanofi-aventis to discover, develop, and commercialize fully human monoclonal antibodies. Contract manufacturing revenue in 2006 related to Regeneron's long-term manufacturing agreement with Merck & Co., Inc., which expired in October 2006. Technology licensing revenue in 2007 related to the Company's license agreements with AstraZeneca and Astellas.

Regeneron recognized contract research and development revenue of $12.6 million in the fourth quarter of 2007 and $47.1 million for the full year 2007 related to the Company's aflibercept collaboration with sanofi-aventis, compared with $9.1 million and $47.8 million, respectively, for the same periods of 2006. Contract research and development revenue from the collaboration consisted of reimbursement of aflibercept development expenses incurred by the Company plus recognition of amounts related to $105.0 million of previously received and deferred non-refundable, up-front payments. Reimbursement of expenses increased to $10.5 million in the fourth quarter of 2007 and to $38.3 million for the full year 2007 from $6.8 million and $36.4 million, respectively, in the comparable periods of 2006, principally due to higher preclinical and clinical development costs and, in the fourth quarter of 2007, higher costs related to the Company's manufacture of aflibercept clinical supplies. With respect to the $105.0 million of up-front payments from sanofi-aventis, $2.1 million was recognized in the fourth quarter of 2007 compared to $2.2 million in the same quarter of 2006, and $8.8 million was recognized in the full year 2007 compared to $11.4 million in the same period of 2006.

Sanofi-aventis also incurs aflibercept development expenses directly and these expenses are increasing because of the growing number of clinical trials sanofi-aventis is overseeing in the aflibercept oncology program. During the term of the aflibercept collaboration, sanofi-aventis pays 100 percent of agreed-upon aflibercept development expenses incurred by both companies. Following commercialization of an aflibercept product, Regeneron, from its 50 percent share of aflibercept profits, will reimburse sanofi-aventis for 50 percent of aflibercept development expenses previously paid by sanofi-aventis.

In connection with the Company's VEGF Trap-Eye collaboration with Bayer HealthCare, the Company received a $75.0 million non-refundable, up-front payment in October 2006 and a $20.0 million milestone payment in August 2007. Through September 30, 2007, all payments received from Bayer HealthCare, including the up-front and milestone payments and cost-sharing reimbursements, were fully deferred and included in deferred revenue. In the fourth quarter of 2007, the Company commenced recognizing previously deferred payments from Bayer HealthCare and cost-sharing of the Company's and Bayer HealthCare's 2007 VEGF Trap-Eye development expenses in the Company's Statement of Operations through a cumulative catch-up. The $75.0 million non-refundable, up-front license payment and $20.0 million milestone payment are being recognized as contract research and development revenue over the related estimated performance period in accordance with Staff Accounting Bulletin No. 104, Revenue Recognition (SAB 104) and Emerging Issues Task Force 00-21, Accounting for Revenue Arrangements with Multiple Deliverables (EITF 00-21). In periods when the Company recognizes VEGF Trap-Eye development expenses that it incurs under the collaboration, the Company also recognizes, as contract research and development revenue, the portion of those VEGF Trap-Eye development expenses that are reimbursable from Bayer HealthCare. In periods when Bayer HealthCare incurs agreed upon VEGF Trap-Eye development expenses that benefit the collaboration and Regeneron, the Company also recognizes, as additional research and development expense, the portion of Bayer HealthCare's VEGF Trap-Eye development expenses that the Company is obligated to reimburse.

In the fourth quarter of 2007, the Company recorded a cumulative catch-up of $35.9 million of contract research and development revenue from Bayer HealthCare, consisting of (i) $15.9 million related to the $75.0 million up-front licensing payment and the $20.0 million milestone payment and (ii) $20.0 million related to the portion of the Company's 2007 VEGF Trap-Eye development expenses that is reimbursable from Bayer HealthCare. In addition, in the fourth quarter of 2007, the Company recorded a cumulative catch-up of $10.6 million of additional research and development expense related to the portion of Bayer HealthCare's 2007 VEGF Trap-Eye development expenses that the Company was obligated to reimburse.

In connection with the Company's antibody collaboration with sanofi-aventis, the Company recognized $4.6 million of contract research and development revenue in the fourth quarter of 2007, which consisted of $3.0 million for reimbursement of the Company's expenses under the collaboration's discovery agreement, $0.7 million for reimbursement of the Company's REGN88 development expenses, and $0.9 million related to the $85.0 million non-refundable, up-front payment, which was deferred upon receipt in December 2007. Contract research and development revenue in connection with the antibody collaboration with sanofi-aventis is being recognized in accordance with SAB 104 and EITF 00-21.

Contract research and development revenue also includes $1.0 million in the fourth quarter of 2007 and $5.5 million for the full year 2007, compared to $0.4 million and $0.5 million, respectively, for the same periods of 2006, in connection with the Company's five-year grant from the National Institutes of Health (NIH), which was awarded to the Company in September 2006 as part of the NIH's Knockout Mouse Project.

In connection with the Company's license agreements with AstraZeneca and Astellas, both of the $20.0 million non-refundable, up-front payments received in February and April 2007, respectively, were deferred and are being recognized as revenue ratably over approximately the first year of each agreement. In the fourth quarter and for the full year 2007, the Company recognized $10.0 million and $28.4 million, respectively, of technology licensing revenue related to these agreements.

Expenses

Total operating expenses for the fourth quarter of 2007 were $76.3 million, 74 percent higher than the same period in 2006, and $239.5 million for the full year 2007, 40 percent higher than for the same period of 2006. Operating expenses included non-cash compensation expense related to employee stock option awards (Stock Option Expense) of $7.5 million in the fourth quarter of 2007 and $28.0 million for the full year 2007, compared

with $5.1 million and $18.4 million, respectively, for the same periods of 2006. The increase in total Stock Option Expense in 2007 was primarily due to the higher fair market value of the Company's Common Stock on the date of annual employee option grants made by the Company in December 2006 in comparison to the fair market value of the Company's Common Stock on the dates of annual employee option grants made in recent prior years.

Research and development (R&D) expenses increased to $64.8 million in the fourth quarter of 2007 from $35.8 million in the comparable quarter of 2006, and to $201.6 million for the full year 2007 from $137.1 million for the same period of 2006. In addition to the impact of Stock Option Expense, as described above, in 2007, the Company incurred higher R&D costs primarily related to additional R&D headcount, clinical development costs for the VEGF Trap-Eye and ARCALYST, research and preclinical development costs for new antibody candidates, and costs to manufacture clinical supplies of ARCALYST and REGN88. Also, as described above, in the fourth quarter of 2007, the Company recorded a cumulative catch-up of $10.6 million of additional research and development expense related to the Company's VEGF Trap-Eye collaboration with Bayer HealthCare.

General and administrative (G&A) expenses increased to $11.4 million in the fourth quarter of 2007 from $7.6 million in the comparable quarter of 2006, and to $37.9 million in the full year 2007 from $25.9 million in the same period of 2006. In addition to the impact of Stock Option Expense, as described above, in 2007, the Company incurred higher compensation expense due, in part, to additional headcount, higher recruitment and related costs associated with expanding the Company's headcount in 2007, and higher fees for various professional services.

Other Income

Investment income decreased to $1.5 million in the fourth quarter of 2007 from $5.5 million in the comparable quarter of 2006, and increased to $20.9 million for the full year 2007 from $16.5 million for the same period of 2006. In the fourth quarter and for the full year 2007, the Company recognized $5.1 million and $5.9 million, respectively, in charges related to certain marketable securities that were determined to be other-than-temporarily impaired in value. For the full-year 2007, the increase in investment income resulted primarily from higher balances of cash and marketable securities due, in part, to the up-front payment received from Bayer HealthCare in October 2006 and the receipt of $174.6 million in net proceeds from the November 2006 public offering of 7.6 million shares of the Company's Common Stock, partly offset by the impairment charges previously described.

About Regeneron Pharmaceuticals

Regeneron is a biopharmaceutical company that discovers, develops, and intends to commercialize therapeutic medicines for the treatment of serious medical conditions. Regeneron has therapeutic candidates in clinical trials for the potential treatment of cancer, eye diseases, and inflammatory diseases, and has preclinical programs in other diseases and disorders.

This news release discusses historical information and includes forward-looking statements about Regeneron and its products, programs, finances, and business, all of which involve a number of risks and uncertainties, such as risks associated with preclinical and clinical development of our drug candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict our ability to continue to develop or commercialize our drug candidates, competing drugs that are superior to our product candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any collaboration agreement, including our agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or to terminate without any product success, risks associated with third party intellectual property, and other material risks. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission (SEC), including its Form 10-K for the year ended December 31, 2006 and Form 10-Q for the quarter ended September 30, 2007. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise unless required by law.

REGENERON PHARMACEUTICALS, INC.
CONDENSED BALANCE SHEETS (Unaudited)
(In thousands)

	December 31, 2007	December 31, 2006
ASSETS		
Cash, restricted cash, and marketable securities	$846,279	$522,859
Receivables	18,320	7,493
Property, plant, and equipment, net	58,304	49,353
Other assets	13,355	5,385
Total assets	$936,258	$585,090
LIABILITIES AND STOCKHOLDERS' EQUITY		
Accounts payable and accrued expenses	$39,232	$21,471
Deferred revenue	236,759	146,995
Notes payable	200,000	200,000
Stockholders' equity	460,267	216,624
Total liabilities and		

stockholders' equity	$936,258 $585,090

REGENERON PHARMACEUTICALS, INC.
CONDENSED STATEMENTS OF OPERATIONS (Unaudited)
(In thousands, except per share data)

	For the three months ended December 31,		For the year ended December 31,	
	2007	2006	2007	2006
Revenues				
Contract research and development	$54,730	$10,110	$96,603	$51,136
Contract manufacturing		236		12,311
Technology licensing	10,000		28,421	
	64,730	10,346	125,024	63,447
Expenses				
Research and development	64,825	35,774	201,613	137,064
Contract manufacturing		430		8,146
General and administrative	11,439	7,628	37,865	25,892
	76,264	43,832	239,478	171,102
Loss from operations	(11,534)	(33,486)	(114,454)	(107,655)
Other income (expense)				
Investment income	1,473	5,525	20,897	16,548
Interest expense	(3,010)	(3,010)	(12,043)	(12,043)
	(1,537)	2,515	8,854	4,505
Net loss before cumulative effect of a change in accounting principle	(13,071)	(30,971)	(105,600)	(103,150)
Cumulative effect of adopting Statement of Financial Accounting Standards No. 123R ("SFAS 123R")				813
Net loss	$(13,071)	$(30,971)	$(105,600)	$(102,337)
Net loss per share amounts, basic and diluted:				
Net loss before cumulative effect of a change in accounting principle	$(0.19)	$(0.51)	$(1.59)	$(1.78)
Cumulative effect of adopting SFAS 123R				0.01
Net loss	$(0.19)	$(0.51)	$(1.59)	$(1.77)
Weighted average shares				

outstanding, basic and diluted	67,754	61,229	66,334	57,970

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SOURCE: Regeneron Pharmaceuticals, Inc.

REGENERON

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

Regeneron and Bayer HealthCare Announce Encouraging 32-Week Follow-Up Results from a Phase 2 Study of VEGF Trap-Eye in Age-Related Macular DegenerationTARRYTOWN, N.Y. & LEVERKUSEN, Germany--(BUSINESS WIRE)--April 28, 2008--Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) and Bayer HealthCare AG today announced that VEGF Trap-Eye dosed on a PRN (as-needed) dosing schedule maintained the statistically significant gain in visual acuity achieved after an initial, 12-week, fixed-dosing phase of a Phase 2 study in the neovascular form of Age-related Macular Degeneration (wet AMD). A full analysis of the 32-week results of the Phase 2 study will be presented today at the 2008 Association for Research in Vision and Ophthalmology (ARVO) meeting in Fort Lauderdale, Florida. The data being reported at the meeting are available on the Regeneron website (www.regeneron.com on the Investor Relations page, under the Presentations heading).

Study results showed that across all dose groups in the study population, the 6.6 mean letter gain in visual acuity achieved versus baseline at the week 16 evaluation visit, following 12 weeks of fixed dosing, was maintained out to week 32 (a 6.7 mean letter gain versus baseline; p less than 0.0001) using a PRN dosing schedule (where dosing frequency was determined by the physician's assessment of pre-specified criteria). The decrease in retinal thickness, an anatomical measure of treatment effect achieved with a fixed-dose schedule was also maintained for all dose groups combined at week 32 (a 137 micron mean decrease versus baseline, p less than0.0001).

In this double-masked, prospective, randomized, multi-center Phase 2 trial, 157 patients were randomized to five dose groups and treated with VEGF Trap-Eye in one eye. Two groups initially received monthly doses of 0.5 or 2.0 milligrams (mg) of VEGF Trap-Eye for 12 weeks and three groups received quarterly doses of 0.5, 2.0, or 4.0 mg of VEGF Trap-Eye (at baseline and week 12). Following the initial 12-week fixed-dose phase of the trial, patients continued to receive therapy at the same dose on a PRN dosing schedule based upon the physician assessment of the need for re-treatment in accordance with pre-specified criteria. Patients were monitored for safety, retinal thickness, and visual acuity. These data represent the week 32 analysis from the 52-week study, which is continuing to follow patients.

Patients receiving monthly doses of VEGF Trap-Eye, either 0.5 or 2.0 mg, for 12 weeks followed by PRN dosing thereafter achieved mean improvements in visual acuity of 8.0 (p less than0.01 versus baseline) and 10.1 letters (p less than0.0001 versus baseline), respectively, and mean decreases in retinal thickness of 141 (p less than0.0001 versus baseline) and 162 microns (p less than0.0001 versus baseline) at week 32, respectively. While PRN dosing also maintained the improvements in retinal thickness and visual acuity achieved versus baseline following a fixed dosing regimen utilizing quarterly dosing at baseline and week 12, the results achieved with a quarterly fixed dosing regimen were generally not as robust as obtained with initial fixed monthly dosing.

VEGF Trap-Eye was generally safe and well tolerated and there were no drug-related serious adverse events. There was one reported case of culture-negative endophthalmitis/uveitis in the study eye, which was deemed not to be drug-related. The most common adverse events were those typically associated with intravitreal injections.

After the last fixed-dose administration at week 12, patients from all dose groups combined required, on average, only one additional injection over the following 20 weeks to maintain the visual acuity gain established during the fixed-dosing period. Notably, 55 percent of the patients who received 2.0 mg monthly for 12 weeks did not require any additional treatment throughout the next 20-week PRN dosing period. Moreover, 97 percent of the patients who received 2.0 mg monthly for 12 weeks did not require re-dosing at the week 16 evaluation visit, indicating that an 8-week dosing schedule may be feasible.

"Due to its high affinity for all isoforms of VEGF-A and PlGF, potent mediators of blood vessel overgrowth in wet AMD, as well as its long residence time in the eye, it is anticipated that VEGF Trap-Eye may be able to be dosed at a frequency less than once monthly, especially on a chronic basis, without compromising visual acuity," stated Quan Dong Nguyen, M.D., M.Sc.,* Assistant Professor of Ophthalmology, Wilmer Ophthalmological Institute, the Johns Hopkins University School of Medicine, Baltimore, MD and a primary investigator in the Phase 2 study. "These emerging Phase 2 clinical data seem to support the concept of durability of VEGF Trap-Eye."

In this study, treatment with VEGF Trap-Eye was associated with a reduction in the size of the choroidal neovascular membrane (CNV), the lesion that is the underlying cause of vision loss due to wet AMD. Patients initially treated with a 0.5 mg or 2.0 mg monthly fixed dose for 12 weeks, followed by PRN dosing thereafter, experienced 1.55 mm(2) and 2.52 mm(2) reductions in mean CNV size at 24 weeks (the most recently available analysis from the independent reading center) versus baseline, respectively. Patients treated initially with fixed quarterly dosing also experienced an overall reduction in CNV size.

"Regression in CNV size is generally not seen when treating wet AMD patients. The reduction in CNV size achieved thus far with VEGF Trap-Eye treatment highlights the potential clinical utility of this investigational treatment in patients suffering from this devastating condition," stated Jason Slakter, M.D., Clinical Professor of Ophthalmology, New York University School of Medicine, New York.

"These study results further increase our confidence in the design of our Phase 3 clinical program for VEGF Trap-Eye in wet AMD," said George D. Yancopoulos, M.D., Ph.D., President of Regeneron Research Laboratories. "These studies are evaluating the clinical efficacy and safety of VEGF Trap-Eye, using a monthly loading dose of 0.5 mg or 2.0 mg for 12 weeks, followed by a nine-month fixed-dosing regimen of 0.5 mg monthly, 2.0 mg monthly, or 2.0 mg every eight weeks. In the second year of the studies, all patients will be dosed on a PRN basis."

About the Phase 3 Program in Wet AMD

Regeneron and Bayer HealthCare initiated a Phase 3 global development program for VEGF Trap-Eye in wet AMD in August 2007. In two Phase 3 trials, the companies are evaluating VEGF Trap-Eye using four- and eight-week dosing intervals in direct comparison with ranibizumab (Lucentis®, a registered trademark of Genentech, Inc.) administered every four weeks according to its label during the first year of the studies. PRN dosing will be evaluated during the second year of each study. The VIEW1 study is currently enrolling patients in the United States and Canada. The VIEW2 study has recently been initiated and will enroll patients in up to 200 centers in Europe, Asia Pacific, Japan, and Latin America. The companies are collaborating on the global development of VEGF Trap-Eye for the treatment of wet AMD, diabetic eye diseases, and other eye diseases and disorders. Bayer HealthCare will market VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of VEGF Trap-Eye. Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States.

About VEGF Trap-Eye

Vascular Endothelial Growth Factor (VEGF) is a naturally occurring protein in the body whose normal role is to trigger formation of new blood vessels (angiogenesis) to support the growth of the body's tissues and organs. It has also been associated with the abnormal growth and fragility of new blood vessels in the eye, which lead to the development of wet AMD. The VEGF Trap-Eye is a fully human, soluble VEGF receptor fusion protein that binds all forms of VEGF-A along with the related Placental Growth Factor (PlGF). VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. Blockade of VEGF, which can prevent abnormal blood vessel formation and vascular leak, has proven beneficial in the treatment of wet AMD and a VEGF inhibitor, ranibizumab, has been approved for treatment of patients with this condition.

About Wet AMD

Age-related Macular Degeneration (AMD) is a leading cause of acquired blindness. Macular degeneration is diagnosed as either dry (nonexudative) or wet (exudative). In wet AMD, new blood vessels grow beneath the retina and leak blood and fluid. This leakage causes disruption and dysfunction of the retina creating blind spots in central vision, and it can account for blindness in wet AMD patients. Wet AMD is the leading cause of blindness for people over the age of 65 in the U.S. and Europe.

About Regeneron Pharmaceuticals, Inc.

Regeneron is a fully integrated biopharmaceutical company that discovers, develops, and commercializes medicines for the treatment of serious medical conditions. In addition to ARCALYST (rilonacept) Injection for Subcutaneous Use, its first commercialized product, Regeneron has therapeutic candidates in clinical trials for the potential treatment of cancer, eye diseases, and inflammatory diseases, and has preclinical programs in other diseases and disorders. Additional information about Regeneron and recent news releases are available on Regeneron's web site at www.regeneron.com.

Forward Looking Statement

This news release discusses historical information and includes forward-looking statements about Regeneron and its products, development programs, finances, and business, all of which involve a number of risks and uncertainties, such as risks associated with preclinical and clinical development of Regeneron's drug candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict Regeneron's ability to continue to develop or commercialize its product and drug candidates, competing drugs that are superior to Regeneron's product and drug candidates, uncertainty of market acceptance of Regeneron's product and drug candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any collaboration agreement, including Regeneron's agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or to terminate without any product success, risks associated with third party intellectual property, and other material risks. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission (SEC), including its Form 10-K for the year ended December 31, 2007. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise unless required by law.

* The assessment made by Dr. Nguyen does not necessarily imply endorsement by the Johns Hopkins University, the Johns Hopkins Hospital, or the Johns Hopkins Medical Institutions.

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SOURCE: Regeneron Pharmaceuticals, Inc.

REGENERON

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

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**Tarrytown, NY and Leverkusen, Germany (August 19, 2008)** – Regeneron Pharmaceuticals, Inc. (Nasdaq: **REGN**) and Bayer HealthCare AG today announced that patients with wet age-related macular degeneration (AMD) receiving VEGF Trap-Eye in a Phase 2 extension study on a PRN (as needed) dosing schedule continued to show highly significant improvements at 52 weeks in the primary and key secondary endpoints of retinal thickness (an anatomic measure of treatment effect) and vision gain. The 12-week primary endpoint results from the fixed-dosing period of the study were presented at the 2007 Retina Society conference in September 2007. The 32-week results of the Phase 2 study were presented at the 2008 Association for Research in Vision and Ophthalmology (ARVO) meeting in Fort Lauderdale, Florida in April 2008. A full analysis of the 52-week results of the Phase 2 study will be presented at the 2008 meeting of the Retina Society on September 26-28, 2008 in Scottsdale, Arizona.

In this double-masked, prospective, randomized, multi-center Phase 2 trial, 157 patients were randomized to five dose groups and treated with VEGF Trap-Eye in one eye. Two groups initially received monthly doses of 0.5 or 2.0 milligrams (mg) of VEGF Trap-Eye (at weeks 0, 4, 8, and 12) and three groups received quarterly doses of 0.5, 2.0, or 4.0 mg of VEGF Trap-Eye (at baseline and week 12). Following the initial 12-week fixed-dosing phase of the trial, patients continued to receive therapy at the same dose on a PRN dosing schedule based upon the physician assessment of the need for re-treatment in accordance with pre-specified criteria. Patients were monitored for safety, retinal thickness, and visual acuity. These data represent the final one-year analysis from the 52-week study.

Patients receiving four monthly doses of VEGF Trap-Eye, either 2.0 or 0.5 mg, for 12 weeks followed by PRN dosing thereafter, achieved mean improvements in visual acuity versus baseline of 9.0 letters ($p<0.0001$) and 5.4 letters ($p=0.085$), respectively, and mean decreases in retinal thickness versus baseline of 143 microns ($p<0.0001$) and 125 microns ($p<0.0001$) at week 52, respectively. During the subsequent PRN dosing phase, patients initially dosed on a 2.0 mg monthly schedule received, on average, only 1.6 additional injections and those initially dosed on a 0.5 mg monthly schedule received, on average, 2.5 injections.

For all dose cohorts combined, there was a 5.3 mean letter gain in visual acuity versus baseline at the week 52 evaluation visit ($p<0.0001$). The mean decrease in retinal thickness for all dose groups combined at week 52 was 130 microns versus baseline ($p<0.0001$). During the week 12 to week 52 PRN dosing period, patients from all dose groups combined received, on average, only two additional injections.

VEGF Trap-Eye was generally well tolerated and there were no drug-related serious adverse events. There was one reported case of culture-negative endophthalmitis/uveitis in the study eye and one arterial thrombotic event, neither of which was deemed to be drug-related. The most common adverse events were those typically associated with intravitreal injections.

"Based upon retinal physicians' feedback, there remains a significant unmet medical need for a treatment for wet AMD that can reliably improve visual acuity over time without the need for monthly intravitreal injections," said George D. Yancopoulos, M.D., Ph.D., President of Regeneron Research Laboratories. "We are excited about these study findings and the potential for VEGF Trap-Eye to fulfill this need pending the results of our ongoing Phase 3 clinical studies."

"The 52-week results underline that VEGF Trap-Eye has the potential to significantly reduce retinal thickness and improve vision," said Dr. Kemal Malik, member of the Bayer HealthCare Executive Committee responsible for product development. "The further development of this compound is important for millions of people worldwide who suffer from this devastating ocular disease."

About the Phase 3 Program in Wet AMD

Regeneron and Bayer HealthCare initiated a Phase 3 global development program for VEGF Trap-Eye in wet AMD in August 2007. In two Phase 3 trials, VIEW 1 and VIEW 2 (**V**EGF Trap-Eye: **I**nvestigation of **E**fficacy and Safety in **W**et Age-related Macular Degeneration), the companies are evaluating VEGF Trap-Eye dosed 0.5 mg every 4 weeks, 2 mg every 4 weeks, or 2 mg every 8 weeks (following three monthly doses) in direct comparison with ranibizumab (Lucentis®, a registered trademark of Genentech, Inc.) administered 0.5 mg every four weeks according to its U.S. label during the first year of the studies. PRN dosing will be evaluated during the second year of each study. The VIEW1 study (http://www.regeneron.com /vegftrap_eye.html) is currently enrolling patients in the United States and Canada and the VIEW2 study (www.view2study.com) is currently enrolling patients in Europe, Asia Pacific, Japan, and Latin America. The companies are collaborating on the global development of VEGF Trap-Eye for the treatment of wet AMD, diabetic eye diseases, and other eye diseases and disorders. Bayer HealthCare will market VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of VEGF Trap-Eye. Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States.

About VEGF Trap-Eye

Vascular Endothelial Growth Factor (VEGF) is a naturally occurring protein in the body whose normal role is to trigger formation of new blood vessels (angiogenesis) to support the growth of the body's tissues and organs. It has also been associated with the abnormal growth and fragility of new blood vessels in the eye, which lead to the development of wet AMD. The VEGF Trap-Eye is a fully human, soluble VEGF receptor fusion protein that binds all forms of VEGF-A along with the related Placental Growth Factor (PlGF). VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. Blockade of VEGF, which can prevent abnormal blood vessel formation and vascular leak, has proven beneficial in the treatment of wet AMD.

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Regeneron is a fully integrated biopharmaceutical company that discovers, develops, and commercializes medicines for the treatment of serious medical conditions. In addition to ARCALYST® (rilonacept) Injection for Subcutaneous Use, its first commercialized product, Regeneron has therapeutic candidates in clinical trials for the potential treatment of cancer, eye diseases, and inflammatory diseases, and has preclinical programs in other diseases and disorders. Additional information about Regeneron and recent news releases are available on Regeneron's web site at www.regeneron.com.

Forward Looking Statement
This news release discusses historical information and includes forward-looking statements about Regeneron and its products, development programs, finances, and business, all of which involve a number of risks and uncertainties, such as risks associated with preclinical and clinical development of Regeneron's drug candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict Regeneron's ability to continue to develop or commercialize its product and drug candidates, competing drugs that are superior to Regeneron's product and drug candidates, uncertainty of market acceptance of Regeneron's product and drug candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any collaboration agreement, including Regeneron's agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or to terminate without any product success, risks associated with third party intellectual property, and other material risks. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission (SEC), including its Form 10-K for the year ended December 31, 2007 and Form 10-Q for the quarter ending June 30, 2008. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise unless required by law.

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REGENERON

Regeneron Reports Full Year and Fourth Quarter 2008 Financial and Operating Results

February 26, 2009
Regeneron Reports Full Year and Fourth Quarter 2008 Financial and Operating ResultsTARRYTOWN, N.Y.--(BUSINESS WIRE)--Feb. 26, 2009-- Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) today announced financial and operating results for the full year and fourth quarter 2008. The Company reported a net loss of $82.7 million, or $1.05 per share (basic and diluted), for the year ended December 31, 2008 compared with a net loss of $105.6 million, or $1.59 per share (basic and diluted), for the same period in 2007. The Company reported a net loss of $31.5 million, or $0.40 per share (basic and diluted), for the fourth quarter of 2008 compared with a net loss of $13.1 million, or $0.19 per share (basic and diluted), for the fourth quarter of 2007. In the fourth quarter of 2007, in connection with the Company's VEGF Trap-Eye collaboration with Bayer HealthCare, the Company recognized a cumulative catch-up of revenue and expenses that reduced the net loss for the quarter by $25.3 million, as described below.

At December 31, 2008, cash, restricted cash, and marketable securities totaled $527.5 million compared with $846.3 million at December 31, 2007. During 2008, the Company retired the full $200 million of its 5.5 percent Convertible Senior Subordinated Notes.

Current Business Highlights

ARCALYST® (rilonacept) – Inflammatory Diseases

The Company shipped $10.7 million of ARCALYST® (rilonacept) Injection for Subcutaneous Use to its distributors in 2008. In February 2008, the Company received marketing approval from the U.S. Food and Drug Administration (FDA) for ARCALYST for the treatment of Cryopyrin-Associated Periodic Syndromes (CAPS), including Familial Cold Auto-inflammatory Syndrome (FCAS) and Muckle-Wells Syndrome (MWS) in adults and children 12 and older. ARCALYST, an interleukin-1 (IL-1) blocker, is the only therapy approved in the United States for patients with CAPS, a group of rare, inherited, auto-inflammatory conditions characterized by life-long, recurrent symptoms of rash, fever/chills, joint pain, eye redness/pain, and fatigue. Intermittent, disruptive exacerbations or flares can be triggered at any time by exposure to cooling temperatures, stress, exercise, or other unknown stimuli.

In March 2008, ARCALYST became available for prescription in the United States, and the Company transitioned the patients who participated in the CAPS pivotal study from clinical study drug to commercial supplies. The Company currently projects shipments of ARCALYST to its distributors to total approximately $20-24 million in 2009.

The Company is in the process of initiating a Phase 3 clinical development program with ARCALYST for the treatment of gout. Two Phase 3 clinical trials will evaluate ARCALYST versus placebo for the prevention of gout flares in patients initiating urate-lowering drug therapy. The Company plans to initiate a Phase 3 clinical trial of ARCALYST for acute gout that will evaluate treatment with ARCALYST alone versus ARCALYST in combination with a non-steroidal anti-inflammatory drug (NSAID) versus an NSAID alone. The Phase 3 clinical development program will also include a separate safety study.

Aflibercept (VEGF Trap) – Oncology

Regeneron and collaborator sanofi-aventis are enrolling patients in four Phase 3 trials that combine aflibercept, an anti-angiogenesis agent, with standard chemotherapy regimens for the treatment of cancer. One trial is evaluating aflibercept as a 2nd line treatment for metastatic colorectal cancer (the VELOUR study) in combination with FOLFIRI (folinic acid (leucovorin), 5-fluorouracil, and irinotecan). A second trial is evaluating aflibercept as a 1st line treatment for metastatic pancreatic cancer in combination with gemcitabine (the VANILLA study). A third trial is evaluating aflibercept as a 1st line treatment for metastatic androgen- independent prostate cancer in combination with docetaxel/prednisone (the VENICE study). The fourth trial is evaluating aflibercept as a 2nd line treatment for metastatic non-small cell lung cancer in combination with docetaxel (the VITAL study). All four trials are studying the current standard of chemotherapy care for the cancer being studied with and without aflibercept. Each of the four Phase 3 trials is over one-third enrolled, and initial data from the Phase 3 program is expected in 2010. In addition, a Phase 2 study of aflibercept in 1st line metastatic colorectal cancer in combination with folinic acid (leucovorin), 5-fluorouracil, and oxaliplatin (the AFFIRM study) began recruiting patients in January 2009.

Aflibercept is also being studied in a Phase 2 single-agent study in advanced ovarian cancer (AOC) patients with symptomatic malignant ascites (SMA). This trial is now fully enrolled and we expect to have initial data from this trial by mid 2009.

VEGF Trap-Eye – Ophthalmologic Diseases

VEGF Trap-Eye is a specially purified and formulated form of VEGF Trap for use in intraocular applications. Regeneron and collaborator Bayer HealthCare are testing VEGF Trap-Eye in a Phase 3 program in patients with the neovascular form of age-related macular degeneration (wet AMD). Regeneron and Bayer HealthCare also initiated a Phase 2 study of VEGF Trap-Eye in patients with diabetic macular edema (DME) in late 2008.

The Phase 3 trials in wet AMD, known as VIEW 1 and VIEW 2 (VEGF Trap: Investigation of Efficacy and Safety in Wet age-related macular degeneration), are comparing VEGF Trap-Eye and ranibizumab (Lucentis®, a registered trademark of Genentech, Inc.), an anti-angiogenic agent approved for use in wet AMD. VIEW 1 is being conducted in North America and VIEW 2 is being conducted in Europe, Asia Pacific, Japan, and Latin America. The VIEW 1 and VIEW 2 trials are both evaluating dosing intervals of four and eight weeks for VEGF Trap-Eye compared with ranibizumab

dosed according to its U.S. label every four weeks over the first year. As needed dosing (PRN) with both agents will be evaluated in the second year of the studies. The VIEW 1 and VIEW 2 trials are expected to complete enrollment in 2009, and initial data are expected in late 2010.

The recently initiated Phase 2 DME study, known as the DA VINCI study, is a double-masked, randomized, controlled trial that is evaluating four different VEGF Trap-Eye regimens versus laser treatment. The study will be enrolling approximately 200 patients in the U.S., Canada, European Union, and Australia. The patients in the study will be treated for 52 weeks followed up by six additional months of safety evaluation. The primary efficacy endpoint is the change in best corrected visual acuity (BCVA) from baseline to week 24.

Monoclonal Antibodies

Regeneron and sanofi-aventis are collaborating on the discovery, development, and commercialization of fully human monoclonal antibodies generated by Regeneron using its *VelocImmune®* technology. The first therapeutic antibodies to enter clinical development under the collaboration are REGN88, an antibody to the interleukin-6 receptor (IL-6R) that is being evaluated in rheumatoid arthritis, and REGN475, an antibody to Nerve Growth Factor (NGF) that is being developed for the treatment of pain. In addition, a Phase 1 trial is in the process of being initiated for REGN421, an antibody to Delta-like ligand-4 (Dll4) that will be evaluated in oncology in patients with advanced malignancies. Over the course of the next several years, the Company and sanofi-aventis plan to advance an average of two to three new fully human antibodies into clinical development each year.

Financial Results

Revenues

Total revenues decreased to $55.8 million in the fourth quarter of 2008 from $64.7 million in the same quarter of 2007 and increased to $238.5 million for the full year 2008 from $125.0 million for the same period of 2007. The Company's revenue was comprised of contract research and development revenue, technology licensing revenue, and net product sales.

Contract Research and Development Revenue

Contract research and development revenue relates primarily to the Company's aflibercept and antibody collaborations with sanofi-aventis and the Company's VEGF Trap-Eye collaboration with Bayer HealthCare. Contract research and development revenue for the three months and years ended December 31, 2008 and 2007, consisted of the following:

	Three months ended December 31,		**Year ended December 31,**	
(In millions)	**2008**	**2007**	**2008**	**2007**
Contract research & development revenue				
Sanofi-aventis	$37.6	$17.2	$154.0	$51.7
Bayer HealthCare	3.0	35.9	31.2	35.9
Other	1.7	1.6	7.0	9.0
Total contract research & development revenue	$42.3	$54.7	$192.2	$96.6

For the three months and years ended December 31, 2008 and 2007, contract research and development revenue from sanofi-aventis consisted of the following:

	Three months ended December 31,		**Year ended December 31,**	
(In millions)	**2008**	**2007**	**2008**	**2007**
Aflibercept:				
Regeneron expense reimbursement	$6.3	$10.5	$35.6	$38.3
Recognition of deferred revenue related to up-front payments	2.5	2.1	8.8	8.8
Total aflibercept	8.8	12.6	44.4	47.1
Antibody:				
Regeneron expense reimbursement	25.5	3.7	97.9	3.7
Recognition of deferred revenue related to up-front payment	2.6	0.9	10.5	0.9
Other	0.7	___	1.2	___
Total antibody	28.8	4.6	109.6	4.6
Total sanofi-aventis contract research & development revenue	$37.6	$17.2	$154.0	$51.7

Sanofi-aventis' reimbursement of Regeneron's aflibercept expenses decreased for the three months and year ended December 31, 2008, compared to the same periods in 2007, primarily due to lower costs related to manufacturing aflibercept clinical supplies.

Revenue under the antibody collaboration increased for the three months and year ended December 31, 2008 compared to the same periods in 2007 due to the initiation of the collaboration in November 2007.

For the three months and years ended December 31, 2008 and 2007, contract research and development revenue from Bayer HealthCare consisted of the following:

	Three months ended December 31,		Year ended December 31,	
(In millions)	**2008**	**2007**	**2008**	**2007**
Cost-sharing of Regeneron VEGF Trap-Eye development expenses	$0.5	$20.0	$18.8	$20.0
Recognition of deferred revenue related to up-front and milestone payments	2.5	15.9	12.4	15.9
Total Bayer HealthCare contract research & development revenue	$3.0	$35.9	$31.2	$35.9

In connection with the Company's VEGF Trap-Eye collaboration with Bayer HealthCare, the Company received a $75.0 million non-refundable, up-front payment in October 2006 and a $20.0 million milestone payment in August 2007. Through September 30, 2007 all payments received from Bayer HealthCare, including the up-front and milestone payments and cost sharing reimbursements, were fully deferred and included in deferred revenue. In the fourth quarter of 2007, the Company commenced recognizing previously deferred payments from Bayer HealthCare and cost sharing of the Company's VEGF Trap-Eye development expenses in the Company's Statement of Operations through a cumulative catch-up. The $75.0 million non-refundable, up-front license payment and $20.0 million milestone payment are being recognized as contract research and development revenue over the related estimated performance period. In periods when the Company recognizes VEGF Trap-Eye development expenses that it incurs under the collaboration, the Company also recognizes, as contract research and development revenue, the portion of those VEGF Trap-Eye development expenses that is reimbursable from Bayer HealthCare. In periods when Bayer HealthCare incurs agreed upon VEGF Trap-Eye development expenses that benefit the collaboration and Regeneron, the Company also recognizes, as additional research and development expense, the portion of Bayer HealthCare's VEGF Trap-Eye development expenses that the Company is obligated to reimburse.

In the fourth quarter of 2007, the Company recorded a cumulative catch-up of $35.9 million of contract research and development revenue from Bayer HealthCare. In addition, in the fourth quarter of 2007, the Company recorded a cumulative catch-up of $10.6 million of additional research and development expense related to the portion of Bayer HealthCare's 2007 VEGF Trap-Eye development expenses that the Company was obligated to reimburse.

Under the terms of the Bayer HealthCare collaboration, in 2008, the first $70.0 million of agreed-upon VEGF Trap-Eye development expenses incurred by the Company and Bayer HealthCare under a global development plan were shared equally, and the Company was solely responsible for up to the next $30.0 million. During the fourth quarter of 2008, Regeneron was solely responsible for most of the collaboration's VEGF Trap-Eye development expenses. As a result, in the fourth quarter of 2008, the portion of the Company's VEGF Trap-Eye development expenses that were reimbursable from Bayer HealthCare, and recognized as contract research and development revenue, amounted to only $0.5 million.

Technology Licensing Revenue

Regeneron has entered into non-exclusive license agreements with AstraZeneca and Astellas that allow those companies to utilize *VelocImmune*® technology in their internal research programs to discover human monoclonal antibodies. Each company made $20.0 million annual, non-refundable payments in each of 2007 and 2008 and agreed to make up to four additional annual payments of $20.0 million, subject to the ability to terminate their agreements after making two such additional payments. Upon receipt, these payments are deferred and are recognized as revenue ratably over approximately the ensuing year of each agreement. Regeneron will also receive a mid-single-digit royalty on sales of any antibodies discovered utilizing *VelocImmune*.

Net Product Sales

The Company shipped $10.7 million of ARCALYST® (rilonacept) to its distributors in 2008 and recorded $3.5 million and $6.3 million in product sales revenue for the three months and year ended December 31, 2008. Revenue and deferred revenue from product sales are recorded net of applicable provisions for prompt pay discounts, product returns, estimated rebates payable under governmental programs (including Medicaid), distributor fees, and other sales-related costs. At December 31, 2008, $4.0 million of ARCALYST net product sales was included in deferred revenue in the Company's financial statements.

Expenses

Total operating expenses for the fourth quarter of 2008 were $90.4 million, 19 percent higher than the same period in 2007, and $328.3 million for the full year 2008, 37 percent higher than for the same period of 2007. Average headcount increased to 903 for the fourth quarter of 2008 compared to 665 for the same period in 2007 and increased to 810 for the full year 2008 from 627 for the full year 2007, due primarily to the Company's expanding research and development activities principally in connection with the Company's antibody collaboration with sanofi-aventis.

Operating expenses included non-cash compensation expense related to employee stock option and restricted stock awards of $7.8 million in the fourth quarter of 2008 and $32.5 million for the full year of 2008, compared with $7.5 million and $28.1 million, respectively, for the same periods of 2007.

Research and development (R&D) expenses increased to $76.3 million in the fourth quarter of 2008 from $64.8 million in the comparable quarter of 2007, and to $278.0 million for the full year 2008 from $201.6 million for the same period of 2007. In the fourth quarter and for the full year of 2008, the Company incurred higher R&D costs primarily related to additional R&D headcount, clinical development costs for ARCALYST and REGN88, research and preclinical development costs associated with our antibody programs, and facility-related costs to support the Company's expanded R&D activities. In addition, for the full year of 2008, the Company incurred higher R&D costs related to clinical development of VEGF Trap-Eye and manufacturing supplies of our drug product candidates, especially our monoclonal antibodies. Also, as described above, commencing in the fourth quarter of 2007, the Company began recognizing as additional R&D expense, the portion of Bayer HealthCare's VEGF Trap-Eye development expenses that the Company is obligated to reimburse.

Selling, general, and administrative (SG&A) expenses increased to $13.5 million in the fourth quarter of 2008 from $11.4 million in the comparable quarter of 2007, and to $49.4 million for the full year 2008 from $37.9 million for the full year 2007. In 2008, the Company incurred $5.2 million of selling expenses related to ARCALYST® (rilonacept) for the treatment of CAPS. In addition, the Company incurred higher compensation expense and recruitment costs associated with expanding the Company's SG&A headcount, higher professional fees related to various general corporate matters, and higher SG&A facility related costs.

Other Income and Expense

Investment income increased to $2.6 million in the fourth quarter of 2008 from $1.5 million in the comparable quarter of 2007, and decreased to $18.2 million for the full year 2008 from $20.9 million for the full year 2007. For the full year 2008, investment income decreased primarily due to lower yields on our cash and marketable securities. The Company recognized charges of $0.2 million and $5.1 million for the fourth quarters of 2008 and 2007, respectively, and $2.5 million and $5.9 million for the full year 2008 and 2007, respectively, related to certain marketable securities that were determined to be other-than-temporarily impaired. For the full year 2008, these charges were partially offset by realized gains of $1.2 million on sales of marketable securities during the year.

During the second and third quarters of 2008, the Company repurchased $82.5 million in principal amount of its 5.5 percent Convertible Senior Subordinated Notes. In connection with the repurchased notes, the Company recognized a $0.9 million loss on early extinguishment of debt. The remaining $117.5 million of these notes were repaid in full upon their maturity in October 2008.

Income Tax Expense

In the fourth quarter of 2008, the Company recognized a $0.7 million income tax benefit, resulting from a provision in the Housing Assistance Tax Act of 2008 that allowed the Company to claim a refund for certain unused pre-2006 research tax credits. For the full year 2008, income tax expense was $2.4 million and consisted primarily of alternative minimum tax, which resulted from the utilization of certain net operating loss carry-forwards, that would otherwise have expired over the next several years, to offset income for tax purposes.

About Regeneron Pharmaceuticals

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This news release discusses historical information and includes forward-looking statements about Regeneron and its products, development programs, finances, and business, all of which involve a number of risks and uncertainties, such as risks associated with preclinical and clinical development of Regeneron's drug candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict Regeneron's ability to continue to develop or commercialize its product and drug candidates, competing drugs that are superior to Regeneron's product and drug candidates, uncertainty of market acceptance of Regeneron's product and drug candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any collaboration agreement, including Regeneron's agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or to terminate without any product success, risks associated with third party intellectual property, and other material risks. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission (SEC), including its Form 10-K for the year ended December 31, 2008. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise unless required by law.

REGENERON PHARMACEUTICALS, INC.
CONDENSED BALANCE SHEETS (Unaudited)
(In thousands)

	December 31, 2008	**December 31, 2007**
ASSETS		
Cash, restricted cash, and marketable securities	$527,461	$846,279
Receivables	35,212	18,320
Property, plant, and equipment, net	87,853	58,304
Other assets	19,512	13,355
Total assets	$670,038	$936,258
LIABILITIES AND STOCKHOLDERS' EQUITY		
Accounts payable, accrued expenses, and other liabilities	$41,261	$39,232
Deferred revenue	209,925	236,759
Notes payable		200,000
Stockholders' equity	418,852	460,267
Total liabilities and stockholders' equity	$670,038	$936,258

REGENERON PHARMACEUTICALS, INC.
CONDENSED STATEMENTS OF OPERATIONS (Unaudited)
(In thousands, except per share data)

	For the three months ended December 31,		For the year ended December 31,	
	2008	2007	2008	2007
Revenues				
Contract research and development	$42,294	$54,730	$192,208	$96,603
Technology licensing	10,000	10,000	40,000	28,421
Net product sales	3,543		6,249	
	55,837	64,730	238,457	125,024
Expenses				
Research and development	76,314	64,825	278,016	201,613
Selling, general, and administrative	13,491	11,439	49,348	37,865
Cost of goods sold	631		923	
	90,436	76,264	328,287	239,478
Loss from operations	(34,599)	(11,534)	(89,830)	(114,454)
Other income (expense)				
Investment income	2,648	1,473	18,161	20,897
Interest expense	(295)	(3,010)	(7,752)	(12,043)
Loss on early extinguishment of debt			(938)	
	2,353	(1,537)	9,471	8,854
Net loss before income tax expense	(32,246)	(13,071)	(80,359)	(105,600)
Income tax expense (benefit)	(728)		2,351	
Net loss	$ (31,518)	$ (13,071)	$ (82,710)	$ (105,600)
Net loss per share amounts, basic and diluted	$ (0.40)	$ (0.19)	$ (1.05)	$ (1.59)
Weighted average shares outstanding, basic and diluted	79,190	67,754	78,827	66,334

Source: Regeneron Pharmaceuticals, Inc.

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REGENERON

Bayer and Regeneron Extend Development Program for VEGF Trap-Eye to Include Central Retinal Vein Occlusion

April 30, 2009

Bayer and Regeneron Extend Development Program for VEGF Trap-Eye to Include Central Retinal Vein OcclusionTwo Phase 3 studies to start in the second half of this year

BERLIN and TARRYTOWN, N.Y., April 30 /PRNewswire-FirstCall/ -- Bayer HealthCare AG and Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) today announced that the companies are extending their global development program for VEGF Trap-Eye, an investigational agent for the treatment of certain eye diseases, to include Central Retinal Vein Occlusion (CRVO). The companies plan to initiate a Phase 3 program evaluating the efficacy and safety of VEGF Trap-Eye in the treatment of CRVO in the second half of this year. CRVO is caused by obstruction of the central retinal vein that that leads to a back up of blood and fluid in the retina, resulting in retinal injury and loss of vision. The retina can also become "ischemic" (starved for oxygen), resulting in the growth of abnormal new blood vessels that can cause further vision loss and more serious complications.

The Phase 3 program in CRVO will consist of two, multinational, one-year clinical studies which have been reviewed with regulatory authorities. These studies will expand the companies' global development collaboration for VEGF Trap-Eye, which already includes two ongoing Phase 3 studies in patients with the neovascular form of Age-related Macular Degeneration (wet AMD) and a Phase 2 study in patients with Diabetic Macular Edema (DME). Enrollment in the wet AMD and DME studies is expected to be completed later this year.

"Although CRVO is a leading cause of blindness, there is currently no treatment available that can be universally considered to be the standard of care, and there is no approved treatment to prevent the loss of vision or improve vision once it is lost," said Dr. Kemal Malik, Head of Global Development and member of the Bayer HealthCare Executive Committee. "Since the underlying biology of CRVO is related to edema and the growth of abnormal new blood vessels that are mediated by vascular endothelial growth factor (VEGF), we are hopeful that VEGF Trap-Eye may help address this significant unmet medical need."

About CRVO

Over 100,000 people in the United States are estimated to suffer from CRVO. CRVO is caused by obstruction of the central retinal vein that leads to a back up of blood and fluid in the retina, resulting in retinal injury and loss of vision. The retina can also become "ischemic" (starved for oxygen), resulting in the growth of new abnormal blood vessels that can cause further vision loss and more serious complications. Release of VEGF contributes to increased vascular permeability in the eye and abnormal new vessel growth. It is believed that anti-VEGF treatment may help decrease vascular permeability and edema and prevent the growth of abnormal new blood vessels in the retina in patients with CRVO.

About the Phase 3 CRVO Program

In the Phase 3 CRVO program for VEGF Trap-Eye, Regeneron and Bayer HealthCare will conduct two identical multinational clinical studies: COPERNICUS (COntrolled Phase 3 Evaluation of Repeated iNtravitreal administration of VEGF Trap-Eye In Central retinal vein occlusion: Utility and Safety) will be led by Regeneron and GALILEO (General Assessment Limiting InfiLtration of Exudates in central retinal vein Occlusion with VEGF Trap-Eye) will be led by Bayer HealthCare. Enrollment will be initiated later in 2009.

Patients in both studies will receive 6 monthly intravitreal injections of either VEGF Trap-Eye at a dose of 2 milligrams (mg) or sham control injections. The primary endpoint of both studies is improvement in visual acuity versus baseline after 6 months of treatment. At the end of the initial 6 months, all patients will be dosed on a PRN (as needed) basis for another 6 months. All patients will be eligible for rescue laser treatment.

About VEGF Trap-Eye

Vascular Endothelial Growth Factor (VEGF) is a naturally occurring protein in the body whose normal role is to trigger formation of new blood vessels (angiogenesis) to support the growth of the body's tissues and organs. It has also been associated with the abnormal growth and fragility of new blood vessels in the eye and vascular permeability and edema. VEGF Trap-Eye is a fully human, soluble VEGF receptor fusion protein that binds all forms of VEGF-A along with the related Placental Growth Factor (PlGF). Investigational VEGF Trap-Eye is a specific blocker of VEGF-A and PlGF that has been demonstrated in preclinical models to bind these growth factors with greater affinity than their natural receptors.

Regeneron and Bayer HealthCare are collaborating on the global development of VEGF Trap-Eye for the treatment of wet AMD, DME, CRVO, and other eye diseases and disorders. Bayer HealthCare will market VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of VEGF Trap-Eye. Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States.

About Regeneron Pharmaceuticals

Regeneron is a fully integrated biopharmaceutical company that discovers, develops, and commercializes medicines for the treatment of serious medical conditions. In addition to ARCALYST® (rilonacept) Injection for Subcutaneous Use, its first commercialized product, Regeneron has therapeutic candidates in clinical trials for the potential treatment of cancer, eye diseases, inflammatory diseases, and pain and has preclinical programs in other diseases and disorders. Additional information about Regeneron and recent news releases are available on Regeneron's web site at www.regeneron.com.

About Bayer HealthCare Pharmaceuticals

Bayer HealthCare Pharmaceuticals Inc. is the U.S.-based pharmaceuticals operation of Bayer HealthCare, an affiliate of Bayer AG. One of the world's

leading, innovative companies in the healthcare and medical products industry, Bayer HealthCare combines the global activities of the Animal Health, Consumer Care, Diabetes Care, and Pharmaceuticals divisions. In the United States, Bayer HealthCare Pharmaceuticals comprises the following business units: Women's Healthcare, Diagnostic Imaging, General Medicine, Hematology/Neurology, and Oncology. The company's aim is to discover and manufacture products that will improve human health worldwide by diagnosing, preventing and treating diseases.

Forward-Looking Statements - Bayer HealthCare AG

This release may contain forward-looking statements based on current assumptions and forecasts made by Bayer Group or subgroup management. Various known and unknown risks, uncertainties and other factors could lead to material differences between the actual future results, financial situation, development or performance of the company and the estimates given here. These factors include those discussed in Bayer's public reports which are available on the Bayer website at www.bayer.com. The company assumes no liability whatsoever to update these forward-looking statements or to conform them to future events or developments.

Forward Looking Statement -- Regeneron Pharmaceuticals, Inc.

This news release discusses historical information and includes forward-looking statements about Regeneron and its products, development programs, finances, and business, all of which involve a number of risks and uncertainties, such as risks associated with preclinical and clinical development of Regeneron's drug candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict Regeneron's ability to continue to develop or commercialize its product and drug candidates, competing drugs that are superior to Regeneron's product and drug candidates, uncertainty of market acceptance of Regeneron's product and drug candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any collaboration agreement, including Regeneron's agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or to terminate without any product success, risks associated with third party intellectual property, and other material risks. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission (SEC), including its Form 10-K for the year ended December 31, 2008. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise unless required by law.

SOURCE Regeneron Pharmaceuticals, Inc.; Bayer HealthCare AG

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http://www.bayer.com /
(REGN)

CO: Regeneron Pharmaceuticals, Inc.; Bayer HealthCare AG; Bayer HealthCare Pharmaceuticals Inc.

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REGENERON

First Patient Enrolled in Regeneron and Bayer HealthCare VEGF Trap-Eye Phase 3 Program in Central Retinal Vein Occlusion

July 23, 2009

First Patient Enrolled in Regeneron and Bayer HealthCare VEGF Trap-Eye Phase 3 Program in Central Retinal Vein OcclusionTARRYTOWN, N.Y.--(BUSINESS WIRE)--Jul. 23, 2009-- Regeneron Pharmaceuticals, Inc. **(NASDAQ:REGN)** today announced that the first patient has been enrolled in the Phase 3 program of VEGF Trap-Eye for the treatment of central retinal vein occlusion (CRVO), a leading cause of blindness in adults. Regeneron received a $20 million milestone payment from Bayer Healthcare that was triggered by the dosing of the first patient in the CRVO program. Regeneron also announced that enrollment in the Phase 2 DA VINCI study of VEGF Trap-Eye in diabetic macular edema (DME) has been completed and data are expected during the first half of 2010.

VEGF Trap-Eye, an investigational drug, is being developed by Regeneron and Bayer HealthCare AG for the potential treatment of eye diseases, including the neovascular form of age-related macular degeneration (wet AMD), DME, and CRVO.

The Phase 3 program in CRVO consists of two multinational, one-year clinical studies. The COPERNICUS (COntrolled Phase 3 Evaluation of Repeated iNtravitreal administration of VEGF Trap-Eye In Central retinal vein occlusion: Utility and Safety) study is being led by Regeneron and the GALILEO (General Assessment Limiting InfiLtration of Exudates in central retinal vein Occlusion with VEGF Trap-Eye) study is being led by Bayer HealthCare. Patients in both studies will receive six monthly intravitreal injections of either VEGF Trap-Eye at a dose of 2 milligrams (mg) or sham control injections. The primary endpoint of both studies is improvement in visual acuity versus baseline after six months of treatment. At the end of the initial six months, patients will be dosed on a PRN (as needed) basis for another six months. All patients will be eligible for rescue laser treatment. Results from both CRVO studies are expected in 2011.

In wet AMD, Regeneron and Bayer Healthcare are evaluating VEGF Trap-Eye in two ongoing Phase 3 studies, known as VIEW 1 and VIEW 2 (VEGF Trap: Investigation of Efficacy and Safety in Wet age-related macular degeneration). Enrollment in these trials is expected to be completed by the end of this year, and data are expected in late 2010.

Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States. Bayer HealthCare has exclusive rights to market VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of VEGF Trap-Eye.

About CRVO

Over 100,000 people in the United States are estimated to suffer from CRVO, a disease for which there is no current treatment that can be considered standard of care. CRVO is caused by obstruction of the central retinal vein that leads to a back up of blood and fluid in the retina, resulting in retinal injury and loss of vision. The retina can also become "ischemic" (starved for oxygen), resulting in the growth of new abnormal blood vessels that can cause further vision loss and more serious complications. Release of VEGF contributes to increased vascular permeability in the eye and abnormal new vessel growth. It is believed that anti-VEGF treatment may help decrease vascular permeability and edema and prevent the growth of abnormal new blood vessels in the retina in patients with CRVO.

About DME

Diabetic Retinopathy (DR) can lead to significant vision impairment and is a major complication of diabetes. Diabetic Macular Edema (DME) is a common complication of DR that involves fluid collection in the macula. DME is the most prevalent cause of moderate visual loss in patients with diabetes.

DME is a leading cause of adult blindness in the developed world. Severe visual loss is caused by a combination of fluid build-up around the retina and the unnatural growth of blood vessels in the back of the eye.

About VEGF Trap-Eye

Vascular Endothelial Growth Factor (VEGF) is a naturally occurring protein in the body whose normal role is to trigger formation of new blood vessels (angiogenesis) to support the growth of the body's tissues and organs. It has also been associated with the abnormal growth and fragility of new blood vessels in the eye and vascular permeability and edema. VEGF Trap-Eye is a fully human, soluble VEGF receptor fusion protein that binds all forms of VEGF-A along with the related Placental Growth Factor (PlGF). Investigational VEGF Trap-Eye is a specific blocker of VEGF-A and PlGF that has been demonstrated in preclinical models to bind these growth factors with greater affinity than their natural receptors.

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Source: Regeneron Pharmaceuticals, Inc.

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REGENERON

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

TARRYTOWN, N.Y., Nov. 19, 2010 /PRNewswire-FirstCall/ -- Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) today announced that it will hold a teleconference and webcast at 8:30 a.m. Eastern Time on Monday, November 22, to discuss results of two Phase 3 studies with VEGF Trap-Eye in Wet Age-related Macular Degeneration, VIEW 1 and VIEW 2, and its VEGF Trap-Eye program. A press release will be issued on Monday prior to the call.

Teleconference/Webcast Details

To participate in the live call on Monday, November 22, at 8:30 a.m. Eastern Time, please dial (877) 390-5538 for domestic callers and (408) 940-3843 for international callers, participant code 27197068. The live conference call is being webcast and it can be accessed on the "Newsroom" page of the Company's web site, www.regeneron.com. The webcast will be available for 30 days following the call.

About Regeneron Pharmaceuticals

Regeneron is a fully integrated biopharmaceutical company that discovers, develops, and commercializes medicines for the treatment of serious medical conditions. In addition to ARCALYST® (rilonacept) Injection for Subcutaneous Use, its first commercialized product, Regeneron has therapeutic candidates in Phase 3 clinical trials for the potential treatment of gout, diseases of the eye (wet age-related macular degeneration and central retinal vein occlusion), and certain cancers. Additional therapeutic candidates developed from proprietary Regeneron technologies for creating fully human monoclonal antibodies are in earlier stage development programs in rheumatoid arthritis and other inflammatory conditions, pain, cholesterol reduction, allergic and immune conditions, and cancer. Additional information about Regeneron and recent news releases are available on Regeneron's web site at www.regeneron.com.

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SOURCE Regeneron Pharmaceuticals, Inc.

News Provided by Acquire Media

REGENERON

Regeneron and Bayer Start Phase 3 Trial to Extend Ophthalmology Research & Development Program for VEGF Trap-Eye in Asia

January 18, 2011

TARRYTOWN, N.Y., BERLIN and SINGAPORE, Jan. 18, 2011 /PRNewswire/ -- Regeneron (Nasdaq: REGN) and Bayer HealthCare today announced initiation of a new Phase 3 clinical trial in collaboration with the Singapore Eye Research Institute (SERI) investigating the efficacy and safety of VEGF Trap-Eye (aflibercept ophthalmic solution) in patients with choroidal neovascularisation (CNV) of the retina as a result of pathologic myopia. The trial has started in Japan and other Asian countries, including China, Korea, Singapore, and Taiwan.

Myopia is one of the most common eye conditions and is highly prevalent in Asian populations, including Singapore where 40% of adults have myopia and nearly 10% have high myopia. Myopic CNV is a complication of high myopia where abnormal blood vessels grow and leak blood and fluid into the retina as a result of degenerative changes in the retinal lining of the eye and is a potentially blinding condition. Currently, there is no well-established treatment for myopic CNV. VEGF Trap-Eye has previously met its primary efficacy endpoint in a Phase 3 trial for neovascular (wet) age-related macular degeneration (AMD).

Collaboration with the Singapore Eye Research Institute (SERI)

SERI has been appointed as the Asian reading center partner for this study. The Singapore Advanced Imaging Laboratory for Ocular Research (SAILOR) will serve as the first reading center for VEGF Trap-Eye studies in the region. SAILOR brings together an inter-disciplinary group of clinician researchers and scientists to collaborate on cutting-edge computer image research. SAILOR is the first clinical translational research unit to be located in Fusionopolis, a research and development complex in Singapore, and serves as a hub of translational research programs in ocular imaging among clinicians, scientists, computer scientists, and other experts. One of the major programs SAILOR has developed is a "tele-ophthalmic ocular imaging platform" to allow transfer and data capture of ocular images for diagnosis and screening. SAILOR will read the images for this myopic CNV trial from the different Asian sites.

"Myopia is a common problem in Singapore and Asia. In particular, myopic CNV, which affects certain groups of people with higher degrees of myopia, may lead to vision loss. There remains uncertainty regarding the best methods of treatment for myopic CNV and this new trial will go towards addressing this clinical need," said Prof. Wong Tien Yin, Director of SERI and Co-Director of SAILOR.

About mCNV

Myopic choroidal neovascularization is a disease of the retina where new, abnormal blood vessels grow into the retina in persons who are severely myopic (typically more than minus six diopters) and have pathological changes in the back of the eye. In myopic patients, the eyeball is too long, which puts strain on the retina and leads to those pathological changes. Anti-VEGF treatment has been shown to be effective in wet age-related macular degeneration, which is also characterised by the growth of new, abnormal blood vessels in the retina. Severe myopia is particularly common in Asia, with some scientists believing that it may be generally more common in Asians than in people from European descent. Myopic CNV (mCNV) is associated with high degrees of myopia and leads to progressive loss of the patient's sight, ending in blindness. In East Asia, the prevalence of myopia is significantly higher than in the West Asia, and seems to have an earlier onset. In Japan, mCNV is the second most common cause of blindness.

About the mCNV Trial

The Phase 3 myopic CNV trial, named MYRROR, will enroll approximately 250 patients and has started in Japan. Other Asian countries, including Singapore, China, Korea, and Taiwan, will join this clinical study throughout the year. Three out of four patients in the trial will receive an injection of VEGF Trap-Eye into the affected eye (and repeated injections on a PRN, as needed, basis, if required). One out of four patients will receive a sham procedure. The clinical outcome of the two treatment groups after 24 weeks will be assessed by a different team of doctors who are unaware of what treatment the patients received. From week 24 onward, sham patients may receive active treatment. The primary outcome measure of the trial is the mean change in vision (best corrected visual acuity) after 24 weeks, compared to baseline. Secondary outcome measures include the percentage of patients who gain or lose certain amounts of letters in the visual test, changes in retinal thickness from baseline, changes in the total mCNV lesion size, and vessel leakage as seen on an angiogram of the affected eye. The study is scheduled to run until June 2013.

About VEGF Trap-Eye

VEGF Trap-Eye is a fully human fusion protein, consisting of soluble VEGF receptors 1 and 2, that binds all forms of VEGF-A along with the related Placental Growth Factor (PlGF). VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. VEGF Trap-Eye is specially purified and contains iso-osmotic buffer concentrations, allowing for injection into the eye.

Bayer HealthCare and Regeneron are collaborating on the global development of VEGF Trap-Eye for the treatment of the neovascular form of age-related macular degeneration (wet AMD), diabetic macular edema (DME), central retinal vein occlusion (CRVO), and other eye diseases and disorders.

In November 2010, Regeneron and Bayer HealthCare announced positive top-line results from two parallel Phase 3 studies in patients with wet AMD, VIEW 1 and VIEW 2. In these trials, all regimens of VEGF Trap-Eye, including VEGF Trap-Eye dosed every two months, successfully met the primary endpoint compared to the current standard of care, ranibizumab dosed every month. The primary endpoint was statistical non-inferiority in the proportion of patients who maintained (or improved) vision over 52 weeks compared to ranibizumab. A generally favorable safety profile was observed for both VEGF Trap-Eye and ranibizumab. The incidence of ocular treatment emergent adverse events was balanced across all four

treatment groups in both studies. There were no notable differences in non-ocular adverse events among the study arms. Bayer HealthCare and Regeneron are planning to submit regulatory applications for marketing approval for the treatment of wet AMD in Europe and the U.S. in the first half of 2011.

Trials in other indications such as CRVO and DME are currently underway or in preparation.

Bayer HealthCare will market VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of VEGF Trap-Eye. Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States.

About Singapore Eye Research Institute (SERI)

SERI is the national research institute for ophthalmic and vision research in Singapore. Serving as the research institute of the Singapore National Eye Centre, and affiliated to the Yong Loo Lin School of Medicine, National University of Singapore, as well the Duke-NUS Graduate Medical School, SERI undertakes vision research in collaboration with local clinical ophthalmic centers and biomedical research institutions, as well as major eye centers and research institutes throughout the world. For further information, kindly visit www.seri.com.sg.

About Regeneron Pharmaceuticals

Regeneron is a fully integrated biopharmaceutical company that discovers, develops, and commercializes medicines for the treatment of serious medical conditions. In addition to ARCALYST® (rilonacept) Injection for Subcutaneous Use, its first commercialized product, Regeneron has therapeutic candidates in Phase 3 clinical trials for the potential treatment of gout, diseases of the eye (wet age-related macular degeneration and central retinal vein occlusion), and certain cancers. Additional therapeutic candidates developed from proprietary Regeneron technologies for creating fully human monoclonal antibodies are in earlier stage development programs in rheumatoid arthritis and other inflammatory conditions, pain, cholesterol reduction, allergic and immune conditions, and cancer. Additional information about Regeneron and recent news releases are available on Regeneron's web site at www.regeneron.com.

About Bayer HealthCare

The Bayer Group is a global enterprise with core competencies in the fields of health care, nutrition and high-tech materials. Bayer HealthCare, a subgroup of Bayer AG with annual sales of EUR 15,988 million (2009), is one of the world's leading, innovative companies in the healthcare and medical products industry and is based in Leverkusen, Germany. The company combines the global activities of the Animal Health, Consumer Care, Medical Care and Pharmaceuticals divisions. Bayer HealthCare's aim is to discover and manufacture products that will improve human and animal health worldwide. Bayer HealthCare has a global workforce of 53.400 employees and is represented in more than 100 countries. Find more information at www.bayerhealthcare.com.

Regeneron Forward Looking Statement

This news release includes forward-looking statements about Regeneron and its products, development programs, finances, and business, all of which involve a number of risks and uncertainties. These include, among others, risks and timing associated with preclinical and clinical development of Regeneron's drug candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict Regeneron's ability to continue to develop or commercialize its product and drug candidates, competing drugs that are superior to Regeneron's product and drug candidates, uncertainty of market acceptance of Regeneron's product and drug candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any license or collaboration agreement, including Regeneron's agreements with the sanofi-aventis Group, Bayer HealthCare, and Astellas to be canceled or terminated without any product success, and risks associated with third party intellectual property. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission (SEC), including its Form 10-K for the year ended December 31, 2009 and Form 10-Q for the quarter ended September 30, 2010. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise, unless required by law.

Bayer Forward-Looking Statements

This release may contain forward-looking statements based on current assumptions and forecasts made by Bayer Group or subgroup management. Various known and unknown risks, uncertainties and other factors could lead to material differences between the actual future results, financial situation, development or performance of the company and the estimates given here. These factors include those discussed in Bayer's public reports which are available on the Bayer website at www.bayer.com. The company assumes no liability whatsoever to update these forward-looking statements or to conform them to future events or developments.

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News Provided by Acquire Media

REGENERON

Regeneron to Webcast Investor Briefing on VEGF Trap-Eye Clinical Program on Sunday, February 13th at 9 am ET

February 9, 2011

TARRYTOWN, N.Y., Feb. 9, 2011 /PRNewswire/ -- Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) today announced that it will webcast an investor briefing on Sunday, February 13 from 9 a.m. to 10:30 a.m. Eastern Time. At the investor briefing, principal investigators from the VEGF Trap-Eye clinical studies will recap presentations from the Bascom Palmer Eye Institute's Angiogenesis, Exudation and Degeneration 2011 meeting being held in Miami, Florida on Saturday, February 12 .

The investigator presentations will provide additional data from the VIEW 1 and VIEW 2 Phase 3 trials in patients with the neovascular form of age-related macular degeneration (wet AMD), the COPERNICUS Phase 3 trial in macular edema due to central retinal vein occlusion (CRVO), and the DA VINCI Phase 2 trial in diabetic macular edema (DME). Regeneron reported positive top-line results from all these trials in the fourth quarter of 2010.

"It's a privilege to be able to release this important collection of VEGF Trap-Eye data at the Bascom Palmer Eye Institute's Eighth Annual Angiogenesis Meeting," said Philip J. Rosenfeld, M.D., Ph.D., Professor of Ophthalmology, University of Miami Miller School of Medicine and Course Co-Director of the Angiogenesis 2011 Meeting. "In particular, the Phase 3 results in wet AMD suggest that the VEGF Trap-Eye has the potential to address an important unmet need of providing optimal vision gain while reducing the burden of intravitreal injections and office visits for patients and their caregivers."

The webcast and slides may be accessed through the Company's web site, www.regeneron.com, on the Investor Relations page (http://investor.regeneron.com). An archived version of the presentation will be available after the live webcast through March 17, 2011.

About VEGF Trap-Eye

VEGF Trap-Eye is a recombinant fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1 that binds all forms of VEGF-A along with the related Placental Growth Factor (PlGF). VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. VEGF Trap-Eye is specially purified and contains iso-osmotic buffer concentrations, allowing for injection into the eye.

Regeneron and Bayer HealthCare are collaborating on the global development of VEGF Trap-Eye for the treatment of wet AMD, DME, CRVO, myopic CNV, and other eye diseases and disorders. The companies plan to submit regulatory applications for marketing approval for VEGF Trap-Eye for the treatment of wet AMD in Europe and the U.S. in the first-half of 2011. Bayer HealthCare will market VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of VEGF Trap-Eye. Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States.

In November 2010, Regeneron and Bayer HealthCare announced positive top-line results from two parallel Phase 3 studies in patients with wet AMD, VIEW 1 and VIEW 2. In these trials, all regimens of VEGF Trap-Eye, including VEGF Trap-Eye dosed every two months, successfully met the primary endpoint compared to the current standard of care, ranibizumab dosed every month. The primary endpoint was statistical non-inferiority in the proportion of patients who maintained (or improved) vision over 52 weeks compared to ranibizumab. A generally favorable safety profile was observed for both VEGF Trap-Eye and ranibizumab. The most frequent ocular adverse events were conjunctival hemorrhage, macular degeneration, eye pain, retinal hemorrhage, and vitreous floaters and were balanced across all treatment groups in both studies. There were no notable differences in non-ocular adverse events among the study arms.

Trials in other indications such as CRVO and DME are currently underway or in preparation.

About Wet Age-Related Macular Degeneration (wet AMD)

Age-related macular degeneration (AMD) is a leading cause of acquired blindness. Macular degeneration is diagnosed as either dry (non-exudative) or wet (exudative). In wet AMD, new blood vessels grow beneath the retina and leak blood and fluid. This leakage causes disruption and dysfunction of the retina creating distortion and/or blind spots in central vision, and it can account for blindness in wet AMD patients. Wet AMD is the leading cause of blindness for people over the age of 65 in the U.S. and Europe. It is estimated that more than 210,000 Americans are newly diagnosed with and treated for wet AMD each year.

About Central Retinal Vein Occlusion (CRVO)

Over 100,000 people in the United States and more than 66,000 people in key European countries are estimated to suffer from central retinal vein occlusion (CRVO). CRVO is caused by obstruction of the central retinal vein that leads to a back up of blood and fluid in the retina. This causes retinal injury and loss of vision. The retina can also become "ischemic" (starved for oxygen), resulting in the growth of new, inappropriate blood vessels that can cause further vision loss and more serious complications. Release of vascular endothelial growth factor (VEGF) contributes to increased vascular permeability in the eye and inappropriate new vessel growth. It is believed that anti-VEGF treatment may help decrease vascular permeability and edema and prevent the inappropriate growth of new blood vessels in the retina in patients with CRVO.

About Diabetic Macular Edema (DME)

Diabetic macular edema (DME) is the most prevalent cause of moderate vision loss in patients with diabetes. DME is a common complication of Diabetic Retinopathy (DR), a disease affecting the blood vessels of the retina. Clinically significant DME is a leading cause of blindness in younger adults (under 50). Clinically significant DME occurs when fluid leaks into the center of the macula, the light-sensitive part of the retina responsible for

sharp, direct vision. Fluid in the macula can cause severe vision loss or blindness.

Approximately 370,000 Americans currently suffer from clinically significant DME, with 95,000 new cases arising each year. According to the American Diabetes Association, more than 18 million Americans currently suffer from diabetes, and many other people are at risk for developing diabetes. With the incidence of diabetes steadily climbing, it is projected that up to 10 percent of all patients with diabetes will develop DME during their lifetime.

About Regeneron Pharmaceuticals

Regeneron is a fully integrated biopharmaceutical company that discovers, develops, and commercializes medicines for the treatment of serious medical conditions. In addition to ARCALYST® (rilonacept) Injection for Subcutaneous Use, its first commercialized product, Regeneron has therapeutic candidates in Phase 3 clinical trials for the potential treatment of gout, diseases of the eye (wet age-related macular degeneration and central retinal vein occlusion), and certain cancers. Additional therapeutic candidates developed from proprietary Regeneron technologies for creating fully human monoclonal antibodies are in earlier stage development programs in rheumatoid arthritis and other inflammatory conditions, pain, cholesterol reduction, allergic and immune conditions, and cancer. Additional information about Regeneron and recent news releases are available on Regeneron's web site at www.regeneron.com.

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REGENERON

February 22, 2011 at 8:00 AM EST

REGENERON SUBMITS BIOLOGICS LICENSE APPLICATION TO FDA FOR VEGF TRAP-EYE FOR TREATMENT OF WET AGE-RELATED MACULAR DEGENERATION

TARRYTOWN, N.Y., Feb. 22, 2011 /PRNewswire/ -- Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) today announced that the company submitted a Biologics License Application (BLA) to the U.S. Food and Drug Administration (FDA) for VEGF Trap-Eye for the treatment of the neovascular form of age-related macular degeneration (wet AMD). Under the Prescription Drug User Fee Act (PDUFA), the goal for a standard review time from submission to FDA action is ten months. Regeneron's submission includes a request for Priority Review, which, if granted, would shorten the FDA's targeted goal for review time under PDUFA to six months.

"There have been significant advances in the treatment of wet AMD in recent years. However, the need for monthly intravitreal injections to obtain optimal vision gains has resulted in a significant burden for physicians, patients, and their caregivers," said Leonard S. Schleifer, M.D., Ph.D., President and Chief Executive Officer of Regeneron. "We are extremely proud to have conducted the largest global Phase 3 clinical program in patients with wet AMD, which demonstrated that patients treated with VEGF Trap-Eye 2 mg every two months, following three loading doses, were able to be dosed with fewer injections over one year without compromising efficacy. We look forward to working closely with the FDA to bring this potentially important new medicine to patients with wet AMD."

The VEGF Trap-Eye BLA is based on the positive results from two Phase 3 trials, the North American VIEW 1 trial and the global VIEW 2 trial. In these trials, all regimens of VEGF Trap-Eye, including VEGF Trap-Eye dosed 2 milligrams (mg) every two months (following three loading doses), successfully met the primary endpoint of non-inferiority, compared to the current standard of care, ranibizumab 0.5 mg dosed every month. The primary endpoint analysis was statistical non-inferiority in the proportion of patients who maintained (or improved) vision over 52 weeks compared to ranibizumab. A generally favorable safety profile was observed for both VEGF Trap-Eye and ranibizumab. The ocular adverse events were balanced across all treatment groups in both studies. There were no notable differences in non-ocular adverse events among the study arms.

About the VIEW Program

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The VIEW (**V**EGF Trap-Eye: **I**nvestigation of **E**fficacy and Safety in **W**et AMD) program consists of two randomized, double-masked, Phase 3 clinical trials evaluating VEGF Trap-Eye in the treatment of the neovascular form of age-related macular degeneration (wet AMD). The VIEW 1 study, which randomized 1217 patients, is being conducted in the United States and Canada by Regeneron under a Special Protocol Assessment (SPA) with the U.S. Food and Drug Administration. The VIEW 2 study, which randomized 1240 patients, is being conducted in Europe, Asia Pacific, Japan, and Latin America by Bayer HealthCare. The study designs are essentially identical. The primary endpoint evaluation was conducted at 52 weeks.

In each of the studies, VEGF Trap-Eye was evaluated for its effect on maintaining and improving vision when dosed as an intravitreal injection on a schedule of 0.5 mg monthly, 2.0 mg monthly, or 2.0 mg every two months (following three monthly loading doses), as compared with intravitreal ranibizumab administered 0.5 mg every month during the first year of the studies.

The primary endpoint of these non-inferiority studies was the proportion of patients treated with VEGF Trap-Eye who maintained visual acuity at the end of one year, compared to ranibizumab patients. Visual acuity was measured as a score based on the total number of letters read correctly on the Early Treatment Diabetic Retinopathy Study (ETDRS) eye chart, a standard chart used in research to measure visual acuity. Maintenance of vision was defined as losing fewer than three lines (equivalent to 15 letters) on the ETDRS eye chart.

The following table summarizes the VIEW 1 and VIEW 2 results for the primary and the first secondary endpoint pre-specified for testing:

	Ranibizumab 0.5mg monthly	**VEGF Trap-Eye 0.5mg monthly**	**VEGF Trap-Eye 2mg monthly**	**VEGF Trap-Eye 2mg every 2 months**
Maintenance of vision* (% patients losing <15 letters) at week 52 versus baseline				
VIEW 1	94.4%	95.9%**	95.1%**	95.1%**
VIEW 2	94.4%	96.3%**	95.6%**	95.6%**
Mean improvement in vision* (letters) at 52 weeks versus baseline (p-value versus ranibizumab 0.5mg monthly)***				
VIEW 1	8.1	6.9 (NS)	10.9 (p<0.01)	7.9 (NS)
VIEW 2	9.4	9.7 (NS)	7.6 (NS)	8.9 (NS)

*Visual acuity was measured as the total number of letters read correctly on the Early Treatment Diabetic Retinopathy Study (ETDRS) eye chart

**Statistically non-inferior based on a non-inferiority margin of 10%, using confidence interval approach (95.1% and 95% for VIEW 1 and VIEW 2, respectively)

*** Test for superiority

NS=not statistically significant

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In the VIEW 1 and VIEW 2 trials, a generally favorable safety profile was observed for both VEGF Trap-Eye and ranibizumab. The incidence of ocular treatment emergent adverse events was balanced across all four treatment groups in both studies, with the most frequent events associated with the injection procedure, the underlying disease, and/or the aging process. The most frequent ocular adverse events were conjunctival hemorrhage, macular degeneration, eye pain, retinal hemorrhage, and vitreous floaters. The most frequent serious non-ocular adverse events were typical of those reported in this elderly population who receive intravitreal treatment for wet AMD; the most frequently reported events were falls, pneumonia, myocardial infarction, atrial fibrillation, breast cancer, and acute coronary syndrome. There were no notable differences among the study arms.

As-needed (PRN) dosing with both agents, with a dose administered at least every three months (but not more often than monthly), is being evaluated during the second year of VIEW 1 and VIEW 2. These studies are part of the global development program for VEGF Trap-Eye being conducted by Regeneron and Bayer HealthCare.

About VEGF Trap-Eye

VEGF Trap-Eye is a fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1 that binds all forms of VEGF-A, along with the related Placental Growth Factor (PlGF). VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. VEGF Trap-Eye is specially purified and contains iso-osmotic buffer concentrations, allowing for injection into the eye.

Regeneron and Bayer HealthCare are collaborating on the development of VEGF Trap-Eye for the treatment of wet AMD, central retinal vein occlusion, diabetic macular edema, myopic choroidal neovascularisation, and other eye diseases and disorders. Bayer HealthCare intends to submit regulatory applications in the first half of 2011 for marketing approval in Europe. If approved by regulatory authorities, Bayer HealthCare will market VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of VEGF Trap-Eye. Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States.

About Wet Age-Related Macular Degeneration (wet AMD)

Age-related macular degeneration (AMD) is a leading cause of acquired blindness. Macular degeneration is diagnosed as either dry (non-exudative) or wet (exudative). In wet AMD, new blood vessels grow beneath the retina and leak blood and fluid. This leakage causes disruption and dysfunction of the retina creating distortion and/or blind spots in central vision, and it can account for blindness in wet AMD patients. Wet AMD is the leading cause of blindness for people over the age of 65 in the U.S. and Europe. It is estimated that more than 210,000 Americans are newly diagnosed with and treated for wet AMD each year.

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loss and more serious complications. Release of vascular endothelial growth factor (VEGF) contributes to increased vascular permeability in the eye and inappropriate new vessel growth. It is believed that anti-VEGF treatment may help decrease vascular permeability and edema and prevent the inappropriate growth of new blood vessels in the retina in patients with CRVO.

About Diabetic Macular Edema (DME)

Diabetic macular edema (DME) is the most prevalent cause of moderate vision loss in patients with diabetes. DME is a common complication of Diabetic Retinopathy (DR), a disease affecting the blood vessels of the retina. Clinically significant DME is a leading cause of blindness in younger adults (under 50). Clinically significant DME occurs when fluid leaks into the center of the macula, the light-sensitive part of the retina responsible for sharp, direct vision. Fluid in the macula can cause severe vision loss or blindness.

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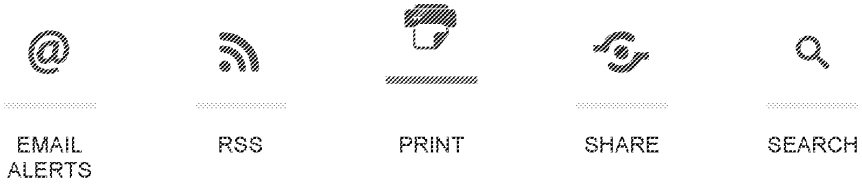
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REGENERON

Regeneron and Bayer Announce Start of Phase 3 Clinical Program in Diabetic Macular Edema

April 8, 2011

Tarrytown, NY, USA, and Berlin, Germany, April 8, 2011 -- Regeneron Pharmaceuticals, Inc. (NASDAQ: **REGN**) and Bayer HealthCare today announced that they have initiated the first of two Phase 3 clinical trials evaluating the efficacy and safety of VEGF Trap-Eye (aflibercept ophthalmic solution), an investigational new agent for the treatment of certain eye diseases, in the treatment of Diabetic Macular Edema (DME). The companies are extending their development program for VEGF Trap-Eye in DME after promising results in the global Phase 2 DME program.

The first Phase 3 trial in DME, named VIVID-DME, is being led by Bayer HealthCare and has started in Australia. The trial will also be conducted in Europe and Japan. A second study led by Regeneron, named VISTA-DME, is expected to begin later in 2011 in the United States, Canada, and other countries.

"Clinically significant DME is a leading cause of vision loss in adults under the age of 50 suffering from diabetes," said Dr. Kemal Malik, Head of Global Development and member of the Bayer HealthCare Executive Committee. "After reporting positive results from our global Phase 3 program (VIEW 1 and VIEW 2 studies) for the treatment of the neovascular form of age-related macular degeneration (wet AMD), we are pleased to start a Phase 3 program with VEGF Trap-Eye in DME which may help to address this significant unmet medical need."

The Phase 3 program in DME expands the companies' global development collaboration for VEGF Trap-Eye. The companies announced positive data for two Phase 3 studies in patients with wet AMD in November 2010 and for the first of two Phase 3 studies in patients with Central Retinal Vein Occlusion (CRVO) in December 2010.

About the Phase 3 DME Program
The VIVID-DME study (**V**EGF Trap-Eye **I**n **V**ision Impairment **D**ue to **DME**) has three study arms. In the first arm, patients will be treated every month with 2 milligrams (mg) of VEGF Trap-Eye. In the second arm, patients will be treated with 2mg of VEGF Trap-Eye every two months after a loading phase of monthly injections. In the third arm, the comparator arm, patients will be treated with macular laser photocoagulation. The primary endpoint is mean change in visual acuity from baseline as measured by the Early Treatment Diabetic Retinopathy Study (ETDRS) eye chart, a standard chart used in research to measure visual acuity. All patients will be followed for three years. The VISTA-DME study (**V**EGF Trap-Eye: **I**nvestigation of **S**afety, **T**reatment effect, and **A**natomic outcomes in **DME**) is expected to begin later in 2011.

About Diabetic Macular Edema (DME)
DME is the most prevalent cause of moderate vision loss in patients with diabetes. DME is a common complication of Diabetic Retinopathy (DR), a disease affecting the blood vessels of the retina. Clinically significant DME is a leading cause of blindness in younger adults (under 50). Clinically significant DME occurs when fluid leaks into the center of the macula, the light-sensitive part of the retina responsible for sharp, direct vision. Fluid in the macula can cause severe vision loss or blindness.

According to figures from the World Health Organization, DME is the second leading cause of blindness in Western industrialized countries. In Europe, about 8% of the population is affected by diabetes. Approximately 370,000 Americans currently suffer from clinically significant DME, with 95,000 new cases arising each year. According to the American Diabetes Association, over 18 million Americans currently suffer from diabetes, and many more are at risk for developing diabetes. The incidence of diabetes is steadily climbing and it is projected that up to 10 percent of all patients with diabetes will develop DME during their lifetime.

About VEGF Trap-Eye
Vascular Endothelial Growth Factor (VEGF) is a naturally occurring protein in the body. Its normal role in a healthy organism is to trigger formation of new blood vessels (angiogenesis) supporting the growth of the body's tissues and organs. However, in certain diseases, such as diabetes, it is also associated with the growth of abnormal new blood vessels in the eye, which exhibit vascular permeability and lead to edema. VEGF Trap-Eye is a fully human, soluble VEGF receptor fusion protein that binds all forms of VEGF-A along with another vascular growth factor, the Placental Growth Factor (PlGF). VEGF Trap-Eye is a specific and highly potent blocker of VEGF-A and PlGF that has been demonstrated in preclinical models to bind these growth factors with greater affinity than their natural receptors. Regeneron and Bayer HealthCare are collaborating on the global development of VEGF Trap-Eye. Bayer HealthCare will market VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of VEGF Trap-Eye. Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States.

Regeneron submitted a Biologics License Application for marketing approval in wet age-related macular degeneration (wet AMD) in the US in February 2011, and Bayer plans to submit a regulatory application outside the US in the first half of 2011. Phase 3 studies in central retinal vein occlusion (CRVO) and in patients with choroidal neovascularisation (CNV) of the retina as a result of pathologic myopia - a major eye disease common in Asia - are currently underway.

About Regeneron Pharmaceuticals
Regeneron is a fully integrated biopharmaceutical company that discovers, develops, and commercializes medicines for the treatment of serious medical conditions. In addition to ARCALYST® (rilonacept) Injection for Subcutaneous Use, its first commercialized product, Regeneron has therapeutic candidates in Phase 3 clinical trials for the potential treatment of gout, diseases of the eye (wet age-related macular degeneration and central retinal vein occlusion), and certain cancers. Additional therapeutic candidates developed from proprietary Regeneron technologies for creating fully human monoclonal antibodies are in earlier stage development programs in rheumatoid arthritis and other inflammatory conditions, pain, cholesterol reduction, allergic and immune conditions, and cancer. Additional information about Regeneron and recent news releases are available on Regeneron's web site at www.regeneron.com.

About Bayer HealthCare

The Bayer Group is a global enterprise with core competencies in the fields of health care, nutrition and high-tech materials. Bayer HealthCare, a subgroup of Bayer AG with annual sales of EUR 16.913 billion (2010), is one of the world's leading, innovative companies in the healthcare and medical products industry and is based in Leverkusen, Germany. The company combines the global activities of the Animal Health, Consumer Care, Medical Care and Pharmaceuticals divisions. Bayer HealthCare's aim is to discover and manufacture products that will improve human and animal health worldwide. Bayer HealthCare has a global workforce of 55.700 employees (Dec 31, 2010) and is represented in more than 100 countries. Find more information at www.bayerhealthcare.com.

Regeneron Forward-Looking Statements
This news release includes forward-looking statements about Regeneron and its products, development programs, finances, and business, all of which involve a number of risks and uncertainties. These include, among others, risks and timing associated with preclinical and clinical development of Regeneron's drug candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict Regeneron's ability to continue to develop or commercialize its product and drug candidates, competing drugs that are superior to Regeneron's product and drug candidates, uncertainty of market acceptance of Regeneron's product and drug candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any license or collaboration agreement, including Regeneron's agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or terminated without any product success, and risks associated with third party intellectual property. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission (SEC), including its Form 10-K for the year ended December 31, 2010. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise, unless required by law.

Bayer Forward-Looking Statements
This release may contain forward-looking statements based on current assumptions and forecasts made by Bayer Group or subgroup management. Various known and unknown risks, uncertainties and other factors could lead to material differences between the actual future results, financial situation, development or performance of the company and the estimates given here. These factors include those discussed in Bayer's public reports which are available on the Bayer website at www.bayer.com. The company assumes no liability whatsoever to update these forward-looking statements or to conform them to future events or developments.

To learn more about Age-related macular degeneration (AMD), please visit:
www.bayerpharma.com.

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REGENERON

FDA Grants Priority Review for VEGF Trap-Eye for the Treatment of Wet Age-Related Macular Degeneration

April 18, 2011

TARRYTOWN, N.Y., April 18, 2011 /PRNewswire/ -- Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) today announced that the U.S. Food and Drug Administration (FDA) has accepted for review the Company's Biologics License Application (BLA) for VEGF Trap-Eye for the treatment of the neovascular form of age-related macular degeneration (wet AMD). The FDA also granted the Company's request for priority review of its BLA. A Priority Review designation is given to drugs that offer major advances in treatment, or provide a treatment where no adequate therapy exists. Under priority review, the target date for an FDA decision on the VEGF Trap-Eye BLA is August 20, 2011.

"We are very pleased that the FDA has chosen to grant priority review to VEGF Trap-Eye. We look forward to working closely with the FDA to achieve our goal of bringing a new treatment option that offers a major advance to patients with age-related macular degeneration," said Leonard S. Schleifer, M.D., Ph.D., President and Chief Executive Officer of Regeneron.

About VEGF Trap-Eye

Vascular Endothelial Growth Factor (VEGF) is a naturally occurring protein in the body. Its normal role in a healthy organism is to trigger formation of new blood vessels (angiogenesis) supporting the growth of the body's tissues and organs. However, in certain diseases, such as diabetes, it is also associated with the growth of abnormal new blood vessels in the eye, which exhibit vascular permeability and lead to edema. VEGF Trap-Eye is a fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1. VEGF Trap-Eye binds all forms of VEGF-A, along with the related Placental Growth Factor (PlGF). VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. VEGF Trap-Eye is specially purified and contains iso-osmotic buffer concentrations, allowing for injection into the eye.

Regeneron and Bayer HealthCare are collaborating on the development of VEGF Trap-Eye for the treatment of wet AMD, central retinal vein occlusion, diabetic macular edema, myopic choroidal neovascularisation, and other eye diseases and disorders. Bayer HealthCare intends to submit a regulatory application outside of the United States in the first half of 2011. If approved by regulatory authorities, Bayer HealthCare will market VEGF Trap-Eye outside the United States, where the companies will share equally in profits from any future sales of VEGF Trap-Eye. Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States.

About Regeneron Pharmaceuticals

Regeneron is a fully integrated biopharmaceutical company that discovers, develops, and commercializes medicines for the treatment of serious medical conditions. In addition to ARCALYST® (rilonacept) Injection for Subcutaneous Use, its first commercialized product, Regeneron has therapeutic candidates in Phase 3 clinical trials for the potential treatment of gout, diseases of the eye (wet age-related macular degeneration and central retinal vein occlusion), and certain cancers. Additional therapeutic candidates developed from proprietary Regeneron technologies for creating fully human monoclonal antibodies are in earlier stage development programs in rheumatoid arthritis and other inflammatory conditions, pain, cholesterol reduction, allergic and immune conditions, and cancer. Additional information about Regeneron and recent news releases are available on Regeneron's web site at www.regeneron.com.

Forward Looking Statements

This news release includes forward-looking statements that involve risks and uncertainties relating to future events and the future financial performance of Regeneron, and actual events or results may differ materially from these forward-looking statements. These statements concern, and these risks and uncertainties include, among others, the nature, timing, and possible success and therapeutic applications of our product candidates and research and clinical programs now underway or planned, the likelihood and timing of possible regulatory approval and commercial launch of our late-stage product candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict our ability to continue to develop or commercialize our product and drug candidates, competing drugs that may be superior to our product and drug candidates, uncertainty of market acceptance of our product and drug candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any license or collaboration agreement, including our agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or terminated without any product success, and risks associated with third party intellectual property. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission, including its Form 10-K for the year ended December 31, 2010. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise, unless required by law.

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SOURCE Regeneron Pharmaceuticals, Inc.

News Provided by Acquire Media

REGENERON

VEGF Trap-Eye Submitted for EU Marketing Authorization for Treatment of Wet Age-Related Macular Degeneration

June 7, 2011

TARRYTOWN, N.Y. and BERLIN, June 7, 2011 /PRNewswire/ -- Regeneron Pharmaceuticals, Inc. (NASDAQ: REGN) and Bayer HealthCare today announced that Bayer HealthCare has submitted an application for marketing authorization in Europe for VEGF Trap-Eye for the treatment of the neovascular form of age-related macular degeneration (wet AMD). Regeneron and Bayer HealthCare are collaborating on the global development of VEGF Trap-Eye for the treatment of wet AMD, central retinal vein occlusion (CRVO), diabetic macular edema (DME), and myopic choroidal neovascularization (mCNV).

"The submission of VEGF Trap-Eye for EU marketing authorization represents a significant milestone in our goal to bring this potentially important new therapy to patients with wet AMD across the globe," said Leonard S. Schleifer, M.D., Ph.D., President and Chief Executive Officer of Regeneron.

The VEGF Trap-Eye submission is based on the positive results from two Phase 3 trials, the VIEW 1 study and the VIEW 2 study. In these trials, all regimens of VEGF Trap-Eye, including 2 mg VEGF Trap-Eye dosed every two months (following three loading doses), successfully met the primary endpoint of non-inferiority, compared to the current standard of care, ranibizumab 0.5 mg dosed every month. The primary endpoint analysis was statistical non-inferiority in the proportion of patients who maintained (or improved) vision over 52 weeks compared to ranibizumab at the dose that is currently known to provide the best possible efficacy. A generally favorable safety profile was observed for both VEGF Trap-Eye and ranibizumab. The ocular adverse events were balanced across all treatment groups in both studies. There were no notable differences in non-ocular adverse events among the study arms.

Regeneron submitted a Biologics License Application (BLA) for marketing approval in wet AMD in the U.S. in February 2011 and received a Priority Review designation.

Bayer HealthCare will market VEGF Trap-Eye outside the United States, where the companies will share equally the profits from any future sales of VEGF Trap-Eye. Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States.

About the VIEW Program

The VIEW (VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD) program consists of two randomized, double-masked, Phase 3 clinical trials evaluating VEGF Trap-Eye in the treatment of the neovascular form of age-related macular degeneration (wet AMD). The VIEW 1 study, which randomized 1,217 patients, is being conducted in the United States and Canada by Regeneron under a Special Protocol Assessment (SPA) with the U.S. Food and Drug Administration. The VIEW 2 study, which randomized 1,240 patients, is being conducted in Europe, Asia Pacific, Japan, and Latin America by Bayer HealthCare. The study designs are essentially identical. The primary endpoint evaluation was conducted at 52 weeks.

In each of the studies, VEGF Trap-Eye was evaluated for its effect on maintaining and improving vision when dosed as an intravitreal injection on a schedule of 0.5 mg monthly, 2 mg monthly, or 2 mg every two months (following three monthly loading doses), as compared with intravitreal ranibizumab administered 0.5 mg every month during the first year of the studies. As-needed (PRN) dosing with both agents, with a dose administered at least every three months (but not more often than monthly) is being evaluated during the second year of each study.

About VEGF Trap-Eye

VEGF Trap-Eye is a fully human fusion protein, consisting of portions of VEGF receptors 1 and 2, that binds all forms of VEGF-A along with the related Placental Growth Factor (PlGF). VEGF Trap-Eye is a specific and highly potent blocker of these growth factors. VEGF Trap-Eye is specially purified and contains iso-osmotic buffer concentrations, allowing for injection into the eye.

Regeneron and Bayer HealthCare are collaborating on the global development of VEGF Trap-Eye for the treatment of the neovascular form of age related macular degeneration (wet AMD), central retinal vein occlusion (CRVO), diabetic macular edema (DME), myopic choroidal neovascularization (mCNV), and other eye diseases and disorders.

Regeneron submitted a Biologics License Application (BLA) for marketing approval in wet AMD in the U.S. in February 2011 and received a Priority Review designation. Under Priority Review, the target date for an FDA decision on the VEGF Trap-Eye BLA is August 20, 2011.

In April 2011, Bayer HealthCare and Regeneron announced the initiation of a Phase 3 program in DME.

Bayer HealthCare will market VEGF Trap-Eye outside the United States, where the companies will share equally the profits from any future sales of VEGF Trap-Eye. Regeneron maintains exclusive rights to VEGF Trap-Eye in the United States.

About Regeneron Pharmaceuticals

Regeneron is a fully integrated biopharmaceutical company that discovers, develops, and commercializes medicines for the treatment of serious medical conditions. In addition to ARCALYST® (rilonacept) Injection for Subcutaneous Use, its first commercialized product, Regeneron has therapeutic candidates in Phase 3 clinical trials for the potential treatment of gout, diseases of the eye (wet age-related macular degeneration, central retinal vein occlusion, and diabetic macular edema), and certain cancers. Additional therapeutic candidates developed from proprietary Regeneron technologies for creating fully human monoclonal antibodies are in earlier stage development programs in rheumatoid arthritis and other inflammatory conditions, pain, cholesterol reduction, allergic and immune conditions, and cancer. Additional information about Regeneron and recent news releases are available on Regeneron's web site at www.regeneron.com.

About Bayer HealthCare

The Bayer Group is a global enterprise with core competencies in the fields of health care, nutrition and high-tech materials. Bayer HealthCare, a subgroup of Bayer AG with annual sales of more than EUR 16.913 billion (2010), is one of the world's leading, innovative companies in the healthcare and medical products industry and is based in Leverkusen, Germany. The company combines the global activities of the Animal Health, Consumer Care, Medical Care and Pharmaceuticals divisions. Bayer HealthCare's aim is to discover and manufacture products that will improve human and animal health worldwide. Bayer HealthCare has a global workforce of 55.700 employees and is represented in more than 100 countries. Find more information at www.bayerhealthcare.com.

Regeneron Forward-Looking Statements

This news release includes forward-looking statements that involve risks and uncertainties relating to future events and the future financial performance of Regeneron, and actual events or results may differ materially from these forward-looking statements. These statements concern, and these risks and uncertainties include, among others, the nature, timing, and possible success and therapeutic applications of Regeneron's product candidates and research and clinical programs now underway or planned, the likelihood and timing of possible regulatory approval and commercial launch of Regeneron's late-stage product candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict Regeneron's ability to continue to develop or commercialize its product and drug candidates, competing drugs that may be superior to Regeneron's product and drug candidates, uncertainty of market acceptance of Regeneron's product and drug candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any license or collaboration agreement, including Regeneron's agreements with the sanofi-aventis Group and Bayer HealthCare, to be canceled or terminated without any product success, and risks associated with third party intellectual property and pending or future litigation relating thereto. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission, including its Form 10-K for the year ended December 31, 2010 and Form 10-Q for the quarter ended March 31, 2011 . Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise, unless required by law.

Bayer Forward-Looking Statements

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SOURCE Regeneron Pharmaceuticals, Inc.

News Provided by Acquire Media

REGENERON

Regeneron Announces EYLEA™ (aflibercept ophthalmic solution) Receives Unanimous Recommendation for Approval for Treatment of Wet AMD from FDA Advisory Committee

June 17, 2011

TARRYTOWN, N.Y., June 17, 2011 /PRNewswire/ -- Regeneron Pharmaceuticals, Inc. (Nasdaq: **REGN**) today announced that the Dermatologic and Ophthalmic Drugs Advisory Committee of the U.S. Food and Drug Administration (FDA) has voted unanimously to recommend that the FDA approve EYLEA™, also known as VEGF Trap-Eye, for the treatment of the neovascular form of age-related macular degeneration (wet AMD) at a dose of 2 milligrams (mg) every eight weeks, following three initial doses given every four weeks.

The committee's recommendation will be considered by the FDA in its review of the Biologics License Application (BLA) for EYLEA, but the committee's recommendation is not binding on the FDA. Regeneron submitted a BLA for marketing approval in wet AMD in the U.S. in February 2011 and received a Priority Review designation. Under Priority Review, the target date for an FDA decision on the EYLEA BLA is August 20, 2011.

"The positive recommendation by the advisory committee is an important step toward providing wet AMD patients with a new treatment option that could potentially reduce the burden that exists with current therapies," said George D. Yancopoulos, M.D., Ph.D., President of Regeneron Research Laboratories. "We look forward to continuing to work with the FDA as it completes its evaluation of the EYLEA BLA."

About EYLEA

Vascular Endothelial Growth Factor (VEGF) is a naturally occurring protein in the body. Its normal role in a healthy organism is to trigger formation of new blood vessels (angiogenesis) supporting the growth of the body's tissues and organs. However, in certain diseases, such as age-related macular degeneration, it is also associated with the growth of abnormal new blood vessels in the eye, which exhibit vascular permeability and lead to edema.

EYLEA (aflibercept ophthalmic solution), also known as VEGF Trap-Eye, is a fully human fusion protein, consisting of portions of VEGF receptors 1 and 2, that binds all forms of VEGF-A along with the related Placental Growth Factor (PlGF). EYLEA is a specific and highly potent blocker of these growth factors. EYLEA is specially purified and contains iso-osmotic buffer concentrations, allowing for injection into the eye.

Regeneron and Bayer HealthCare are collaborating on the global development of EYLEA for the treatment of the neovascular form of age-related macular degeneration (wet AMD), central retinal vein occlusion (CRVO), diabetic macular edema (DME), and other eye diseases and disorders. Bayer submitted an application for marketing authorization in Europe in wet AMD in June 2011.

The EYLEA wet AMD regulatory submissions are based on the positive results from two Phase 3 trials, the VIEW 1 study and the VIEW 2 study. In these trials, all regimens of EYLEA, including 2 milligrams (mg) of EYLEA dosed every two months (following three loading doses), successfully met the primary endpoint of non-inferiority compared to the current standard of care, ranibizumab 0.5 mg dosed every month. The primary endpoint analysis was statistical non-inferiority in the proportion of patients who maintained (or improved) vision over 52 weeks compared to ranibizumab. A generally favorable safety profile was observed for both EYLEA and ranibizumab. The most frequent ocular adverse events were conjunctival hemorrhage, macular degeneration, eye pain, retinal hemorrhage, and vitreous floaters.

Bayer HealthCare will market EYLEA™ outside the United States, where the companies will share equally the profits from any future sales of EYLEA. Regeneron maintains exclusive rights to EYLEA in the United States.

About Regeneron Pharmaceuticals

Regeneron is a fully integrated biopharmaceutical company that discovers, develops, and commercializes medicines for the treatment of serious medical conditions. In addition to ARCALYST® (rilonacept) Injection for Subcutaneous Use, its first commercialized product, Regeneron has therapeutic candidates in Phase 3 clinical trials for the potential treatment of gout, diseases of the eye (wet age-related macular degeneration, central retinal vein occlusion, and diabetic macular edema), and certain cancers. Additional therapeutic candidates developed from proprietary Regeneron technologies for creating fully human monoclonal antibodies are in earlier stage development programs in rheumatoid arthritis and other inflammatory conditions, pain, cholesterol reduction, allergic and immune conditions, and cancer. Additional information about Regeneron and recent news releases are available on Regeneron's web site at www.regeneron.com.

Regeneron Forward Looking Statement

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SOURCE Regeneron Pharmaceuticals, Inc.

News Provided by Acquire Media

REGENERON

August 17, 2011

Regeneron Announces Clinical Presentations at ASRS 2011 Annual Meeting

TARRYTOWN, N.Y., Aug. 17, 2011 /PRNewswire/ -- Regeneron Pharmaceuticals, Inc. (Nasdaq: REGN) today announced that clinical data from four separate clinical studies of EYLEA™ (aflibercept injection) will be presented at the upcoming American Society of Retina Specialists (ASRS) meeting on Sunday, August 21 and Monday, August 22, 2011 in Boston, Massachusetts.

The presentations are:

- "Analysis of 2,457 Patients in the Phase 3 VIEW 1 and VIEW 2 Studies Comparing VEGF Trap-Eye and Ranibizumab in Neovascular AMD" will be presented by Jeffrey S. Heier, M.D. on Sunday, August 21 at 8:21 a.m.

- "One-year Results of the DA VINCI Study of VEGF Trap-Eye in DME" will be presented by Diana V. Do, M.D. on Sunday, August 21 at 2:48 p.m.

- "The 6-Month (Primary Endpoint) Results of the Phase 3 GALILEO Study: VEGF Trap-Eye in CRVO" will be presented by Jean-Francois Korobelnik, M.D. on Monday, August 22 at 8:20 a.m.

- "Trap-Eye in CRVO: 1-year Results of the Phase 3 COPERNICUS Study" will be presented by W. Lloyd Clark, M.D. on Monday, August 22 at 8:28 a.m.

About EYLEA™ (aflibercept injection)

Vascular Endothelial Growth Factor (VEGF) is a naturally occurring protein in the body. Its normal role in a healthy organism is to trigger formation of new blood vessels (angiogenesis) supporting the growth of the body's tissues and organs. However, in certain diseases, such as age-related macular degeneration, it is also associated with the growth of abnormal new blood vessels in the eye, which exhibit vascular permeability and lead to edema.

EYLEA, also known as VEGF Trap-Eye, is a fully human fusion protein, consisting of portions of VEGF receptors 1 and 2, that binds all forms of VEGF-A along with the related Placental Growth Factor (PlGF). EYLEA is a specific and highly potent blocker of these growth factors. EYLEA is specially purified and contains iso-osmotic buffer concentrations, allowing for injection into the eye.

Regeneron and Bayer HealthCare are collaborating on the global development of EYLEA for the treatment of neovascular age-related macular degeneration (wet AMD), central retinal vein occlusion (CRVO), diabetic macular edema (DME), and other eye diseases and disorders. Bayer submitted an application for marketing authorization in Europe in wet AMD in June 2011.

Bayer HealthCare will market EYLEA™ (aflibercept injection) outside the United States, where the companies will share equally the profits from any future sales of EYLEA. Regeneron maintains exclusive rights to EYLEA in the United States.

About Regeneron Pharmaceuticals

Regeneron is a fully integrated biopharmaceutical company that discovers, develops, and commercializes medicines for the treatment of serious medical conditions. In addition to ARCALYST® (rilonacept) Injection for Subcutaneous Use, its first commercialized product, Regeneron has therapeutic candidates in Phase 3 clinical trials for the potential treatment of gout, diseases of the eye (wet age-related macular degeneration, central retinal vein occlusion, and diabetic macular edema), and certain cancers. Additional therapeutic candidates developed from proprietary Regeneron technologies for creating fully human monoclonal antibodies are in earlier stage development programs in rheumatoid arthritis and other inflammatory conditions, pain, cholesterol reduction, allergic and immune conditions, and cancer. Additional information about Regeneron and recent news releases are available on Regeneron's web site at www.regeneron.com.

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SOURCE Regeneron Pharmaceuticals, Inc.

News Provided by Acquire Media

REGENERON

Regeneron Announces FDA Approval of EYLEA™ (aflibercept) Injection for the Treatment of Wet Age-Related Macular Degeneration: CORRECTED

November 18, 2011

In the news release, Regeneron Announces FDA Approval of EYLEA™ (aflibercept) Injection for the Treatment of Wet Age-Related Macular Degeneration, issued 18-Nov-2011 by Regeneron Pharmaceuticals, Inc. over PR Newswire, the third paragraph, second sentence, should read "EYLEA offers the potential of achieving the efficacy we've come to expect from current anti-VEGF agents, but with less frequent injections and monitoring." The complete, corrected release follows:

TARRYTOWN, N.Y., Nov. 18, 2011 /PRNewswire/ -- Regeneron Pharmaceuticals, Inc. (Nasdaq: **REGN**) today announced that the U.S. Food and Drug Administration (FDA) has approved EYLEA (aflibercept) Injection, known in the scientific literature as VEGF Trap-Eye, for the treatment of patients with neovascular (wet) Age-related Macular Degeneration (AMD) at a recommended dose of 2 milligrams (mg) every four weeks (monthly) for the first 12 weeks, followed by 2 mg every eight weeks (2 months).

The approval of EYLEA was granted under a Priority Review, a designation that is given to drugs that offer major advances in treatment, or provide a treatment where no adequate therapy exists. This approval was based upon the results of two Phase 3 clinical studies. In these studies, EYLEA dosed every eight weeks, following three initial monthly injections, was clinically equivalent to the standard of care, Lucentis® (ranibizumab injection) dosed every four weeks, as measured by the primary endpoint of maintenance of visual acuity (less than 15 letters of vision loss on an eye chart) over 52 weeks. The most common adverse reactions (frequency of 5% or more) reported in patients receiving EYLEA were conjunctival hemorrhage, eye pain, cataract, vitreous detachment, vitreous floaters, and increased intraocular pressure. The adverse event profile was similar to that seen with ranibizumab.

"The approval of EYLEA offers a much needed new treatment option for patients with wet AMD," said Jeffrey Heier, M.D., a clinical ophthalmologist and retinal specialist at Ophthalmic Consultants of Boston, Assistant Professor of Ophthalmology at Tufts School of Medicine, and Chair of the Steering Committee for the VIEW 1 trial. "EYLEA offers the potential of achieving the efficacy we've come to expect from current anti-VEGF agents, but with less frequent injections and monitoring. This may reduce the need for costly and time-consuming monthly office visits for patients and their caregivers."

"This approval is an important step forward for Regeneron and for patients suffering with wet AMD, the most common cause of blindness in the U.S. in older adults," said Leonard S. Schleifer, M.D., Ph.D., President and Chief Executive Officer of Regeneron. "We thank the patients and clinical investigators who participated in our clinical studies, the FDA, and the Regeneron employees who helped make this day possible. Now that EYLEA is approved, we plan to make EYLEA available to patients within the next few days."

About EYLEA™ (aflibercept) Injection

Vascular Endothelial Growth Factor (VEGF) is a naturally occurring protein in the body. Its normal role in a healthy organism is to trigger formation of new blood vessels (angiogenesis) supporting the growth of the body's tissues and organs. However, in certain diseases, such as wet age-related macular degeneration, it is also associated with the growth of abnormal new blood vessels in the eye, which exhibit abnormal increased permeability that leads to edema. Scarring and loss of fine-resolution central vision often results.

EYLEA, known in the scientific literature as VEGF Trap-Eye, is a recombinant fusion protein, consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1 and formulated as an iso-osmotic solution for intravitreal administration. EYLEA acts as a soluble decoy receptor that binds VEGF-A and placental growth factor (PlGF) and thereby can inhibit the binding and activation of these cognate VEGF receptors.

EYLEA is indicated for the treatment of patients with neovascular age-related macular degeneration (wet AMD). EYLEA is contraindicated in patients with ocular or periocular infections, active intraocular inflammation, or known hypersensitivity to aflibercept or to any of the excipients in EYLEA.

The recommended dose for EYLEA is 2 mg administered by intravitreal injection every four weeks (monthly) for the first 12 weeks (3 months), followed by 2 mg once every eight weeks (2 months). Although EYLEA may be dosed as frequently as 2 mg every four weeks (monthly), additional efficacy was not demonstrated when EYLEA was dosed every four weeks compared to every eight weeks.

There is a potential risk of arterial thromboembolic events (ATEs) following use of intravitreal VEGF inhibitors, including EYLEA, defined as nonfatal stroke, nonfatal myocardial infarction, or vascular death (including deaths of unknown cause). The incidence of ATEs with EYLEA in clinical trials was low (1.8%).

Serious adverse reactions related to the injection procedure have occurred in less than 0.1% of intravitreal injections with EYLEA and include endophthalmitis, traumatic cataract, and increased intraocular pressure.

About the VIEW 1 and VIEW 2 Clinical Studies

The safety and efficacy of EYLEA were assessed in two randomized, multi-center, double-masked, active-controlled studies in patients with wet AMD. A total of 2412 patients were treated and evaluable for efficacy (1817 with EYLEA) in the two studies (VIEW 1 and VIEW 2). In each study, patients were randomly assigned in a 1:1:1:1 ratio to one of four dosing regimens: 1) EYLEA administered 2 mg every eight weeks following three initial

monthly doses (EYLEA 2Q8); 2) EYLEA administered 2 mg every four weeks (EYLEA 2Q4); 3) EYLEA 0.5 mg administered every four weeks (EYLEA 0.5Q4); and 4) ranibizumab administered 0.5 mg every four weeks (ranibizumab 0.5Q4). Patient ages ranged from 49 to 99 years with a mean of 76 years.

In both studies, the primary efficacy endpoint was the proportion of patients who maintained vision, defined as losing fewer than 15 letters of visual acuity at week 52 compared to baseline. Data are available through week 52. Both the EYLEA™ (aflibercept) Injection 2Q8 and 2Q4 dosing groups were shown to have efficacy that was clinically equivalent to the ranibizumab 0.5Q4 group for the primary endpoint.

Select results of the VIEW 1 and VIEW 2 studies as described in the full Prescribing Information for the EYLEA 2 mg every four weeks and EYLEA 2 mg every eight weeks dosing groups as compared to ranibizumab dosed monthly group are shown below.

Efficacy Outcomes at Week 52 (Full Analysis Set with LOCF) in VIEW 1 and VIEW 2 Studies

	VIEW 1			**VIEW 2**		
	EYLEA 2 mg Q8 weeks(a)	EYLEA 2 mg Q4 weeks	ranibizu-mab 0.5 mg Q4 weeks	EYLEA 2 mg Q8 weeks(a)	EYLEA 2 mg Q4 weeks	ranibizu-mab 0.5 mg Q4 weeks
Full Analysis Set	N=301	N=304	N=304	N=306	N=309	N=291
Efficacy Outcomes						
Proportion of patients who maintained visual acuity (%) (						

REGENERON

Regeneron and Bayer Initiate Phase 3 Clinical Program for the Treatment of Wet Age-Related Macular Degeneration in China

November 28, 2011

TARRYTOWN, N.Y. and BERLIN, Nov. 28, 2011 /PRNewswire/ -- Regeneron Pharmaceuticals, Inc. (NASDAQ: REGN) and Bayer HealthCare today announced that they have initiated a Phase 3 clinical trial evaluating the efficacy and safety of EYLEA™ (aflibercept) Injection in the neovascular form of age-related macular degeneration (wet AMD) in China.

The new trial, named SIGHT, will include approximately 300 patients and will be the largest retinal trial conducted in China. SIGHT is being led by Bayer.

"Currently, only photodynamic therapy with verteporfin is approved as a treatment for wet AMD in China, and it is only approved for the subpopulation of patients with predominantly classic wet AMD," said Kemal Malik, M.D., Head of Global Development and member of the Bayer HealthCare Executive Committee. "After reporting positive data from our large VIEW program in wet AMD, we look forward to potentially bringing this new treatment to patients with wet AMD in China."

About the SIGHT Program

The SIGHT (VEGF Trap-Eye: Investigation of Efficacy and Safety in Chinese patients with wet AMD) program consists of a randomized, double-masked, Phase 3 clinical trial evaluating EYLEA (known in the scientific literature as VEGF Trap-Eye) in the treatment of the neovascular form of age-related macular degeneration (wet AMD). EYLEA will be evaluated for its effect on improving and maintaining vision when dosed as an intravitreal injection on a schedule of 2 milligrams (mg) every two months (following three initial monthly doses), as compared with Photodynamic Therapy (PDT) with verteporfin. After assessment of the primary endpoint at week 28, all patients, including those on PDT, will receive EYLEA treatment until the end of the study at week 52. The SIGHT study plans to randomize 300 patients.

About Wet AMD

Age-related Macular Degeneration (AMD) is a leading cause of acquired blindness. Macular degeneration is diagnosed as either dry (non-exudative) or wet (exudative). In wet AMD, new blood vessels grow beneath the retina and leak blood and fluid. This leakage causes disruption and dysfunction of the retina creating blind spots in central vision, and it can account for blindness in wet AMD patients. Wet AMD is the leading cause of blindness for people over the age of 65 in the U.S. and Europe. In China, there were an estimated 540,000 newly diagnosed wet AMD patients over the age of 50 in 2010.

About EYLEA™ (aflibercept) Injection For Intravitreal Injection

Vascular Endothelial Growth Factor (VEGF) is a naturally occurring protein in the body. Its normal role in a healthy organism is to trigger formation of new blood vessels (angiogenesis) supporting the growth of the body's tissues and organs. However, in certain diseases, such as wet age-related macular degeneration, it is also associated with the growth of abnormal new blood vessels in the eye, which exhibit abnormal increased permeability that leads to edema. Scarring and loss of fine-resolution central vision often results.

EYLEA, known in the scientific literature as VEGF Trap-Eye, is a recombinant fusion protein, consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1 and formulated as an iso-osmotic solution for intravitreal administration. EYLEA acts as a soluble decoy receptor that binds VEGF-A and placental growth factor (PlGF) and thereby can inhibit the binding and activation of these cognate VEGF receptors.

IMPORTANT PRESCRIBING INFORMATION

In this U.S. EYLEA is indicated for the treatment of patients with neovascular age-related macular degeneration (wet AMD).

The recommended dose for EYLEA is 2 mg administered by intravitreal injection every four weeks (monthly) for the first 12 weeks (3 months), followed by 2 mg once every eight weeks (2 months). Although EYLEA may be dosed as frequently as 2 mg every four weeks (monthly), additional efficacy was not demonstrated when EYLEA was dosed every four weeks compared to every eight weeks.

IMPORTANT SAFETY INFORMATION

EYLEA is contraindicated in patients with ocular or periocular infections, active intraocular inflammation, or known hypersensitivity to aflibercept or to any of the excipients in EYLEA.

Intravitreal injections, including those with EYLEA, have been associated with endophthalmitis and retinal detachments. Proper aseptic injection technique must always be used when administering EYLEA. Patients should be instructed to report any symptoms suggestive of endophthalmitis or retinal detachment without delay and should be managed appropriately.

Acute increases in intraocular pressure have been seen within 60 minutes of intravitreal injection, including with EYLEA. Sustained increases in intraocular pressure have also been reported after repeated intravitreal dosing with VEGF inhibitors. Intraocular pressure and the perfusion of the optic nerve head should be monitored and managed appropriately.

There is a potential risk of arterial thromboembolic events (ATEs) following use of intravitreal VEGF inhibitors, including EYLEA, defined as nonfatal

stroke, nonfatal myocardial infarction, or vascular death (including deaths of unknown cause). The incidence of ATEs with EYLEA in clinical trials was low (1.8%).

Serious adverse reactions related to the injection procedure have occurred in less than 0.1% of intravitreal injections with EYLEA including endophthalmitis, traumatic cataract, and increased intraocular pressure.

The most common adverse reactions (greater than or equal to 5%) reported in patients receiving EYLEA were conjunctival hemorrhage, eye pain, cataract, vitreous detachment, vitreous floaters, and increased intraocular pressure.

Please see the full Prescribing Information for EYLEA, available online at www.regeneron.com/EYLEA.fpi.pdf.

About the EYLEA™ (aflibercept) Injection Global Collaboration

Regeneron is collaborating with Bayer HealthCare on the global development of EYLEA. Bayer submitted an application for marketing authorization in Europe for wet AMD in June 2011.

Bayer HealthCare will market EYLEA outside the United States, where the companies will share equally the profits from any future sales of EYLEA. Regeneron maintains exclusive rights to EYLEA in the United States.

About Regeneron Pharmaceuticals

Regeneron is a fully integrated biopharmaceutical company that discovers, invents, develops, manufactures, and commercializes medicines for the treatment of serious medical conditions. Regeneron markets two products, ARCALYST® (rilonacept) Injection For Subcutaneous Use and EYLEA™ (aflibercept) Injection. Regeneron also has completed several Phase 3 studies and is conducting an additional Phase 3 clinical trial for the product candidate ZALTRAP® (aflibercept) Concentrate for Intravenous Infusion. Additional therapeutic candidates developed from proprietary Regeneron technologies for creating fully human monoclonal antibodies are in earlier stage development programs in rheumatoid arthritis and other inflammatory conditions, pain, cholesterol reduction, allergic and immune conditions, and cancer. Additional information about Regeneron and recent news releases are available on the Regeneron web site at www.regeneron.com.

About Bayer HealthCare

The Bayer Group is a global enterprise with core competencies in the fields of health care, nutrition and high-tech materials. Bayer HealthCare, a subgroup of Bayer AG with annual sales of EUR 16.913 billion (2010), is one of the world's leading, innovative companies in the healthcare and medical products industry and is based in Leverkusen, Germany. The company combines the global activities of the Animal Health, Consumer Care, Medical Care and Pharmaceuticals divisions. Bayer HealthCare's aim is to discover and manufacture products that will improve human and animal health worldwide. Bayer HealthCare has a global workforce of 55,700 employees (Dec 31, 2010) and is represented in more than 100 countries. Find more information at www.bayerhealthcare.com.

To learn more about wet Age-related Macular Degeneration (AMD), please visit www.bayerpharma.com/en/AMD

Regeneron Forward-Looking Statement

This news release includes forward-looking statements that involve risks and uncertainties relating to future events and the future performance of Regeneron, and actual events or results may differ materially from these forward-looking statements. These statements concern, and these risks and uncertainties include, among others, the nature, timing, and possible success and therapeutic applications of EYLEA and Regeneron's product candidates and research and clinical programs now underway or planned, the likelihood and timing of possible regulatory approval and commercial launch of Regeneron's late-stage product candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict Regeneron's ability to continue to develop or commercialize EYLEA and other products and drug candidates, competing drugs that may be superior to EYLEA and Regeneron's products and drug candidates, uncertainty of market acceptance of EYLEA and Regeneron's products and drug candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any license or collaboration agreement, including Regeneron's agreements with Sanofi and Bayer HealthCare, to be canceled or terminated without any product success, and risks associated with third party intellectual property and pending or future litigation relating thereto. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission, including its Form 10-K for the year ended December 31, 2010 and Form 10-Q for the quarter ended September 30, 2011. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise, unless required by law.

Bayer Forward-Looking Statement

This release may contain forward-looking statements based on current assumptions and forecasts made by Bayer Group or subgroup management. Various known and unknown risks, uncertainties and other factors could lead to material differences between the actual future results, financial situation, development or performance of the company and the estimates given here. These factors include those discussed in Bayer's public reports which are available on the Bayer website at www.bayer.com. The company assumes no liability whatsoever to update these forward-looking statements or to conform them to future events or developments.

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SOURCE Regeneron Pharmaceuticals, Inc.

News Provided by Acquire Media

REGENERON

Two Year Results of Phase 3 Studies with EYLEA™ (aflibercept) Injection in wet AMD Show Sustained Improvement in Visual Acuity

December 5, 2011

Patients in the EYLEA 2mg every eight week group achieved visual acuity gains similar to ranibizumab with 5 fewer injections, on average, over two years
Patients who required the most intense therapy received, on average, 1.4 fewer injections in the EYLEA 2mg every eight week group compared to ranibizumab in the second year

TARRYTOWN, N.Y. and BERLIN, Dec. 5, 2011 /PRNewswire/ -- Regeneron Pharmaceuticals, Inc. (NASDAQ: REGN) and Bayer HealthCare today announced that in an integrated analysis of two parallel Phase 3 studies (VIEW 1 and VIEW 2) in patients with the neovascular form of age-related macular degeneration (wet AMD), patients treated with EYLEA™ (aflibercept) Injection For Intravitreal Injection showed a sustained improvement in visual acuity at 96 weeks versus baseline. The 52-week results (primary analyses) from these studies have previously been reported.

During the first year of the VIEW 1 and VIEW 2 studies, patients were treated with three different dosing regimens of EYLEA, 0.5 milligram (mg) every four weeks, 2mg every four weeks, and 2mg every eight weeks (following three initial monthly injections), compared to ranibizumab 0.5mg every four weeks. The EYLEA 2mg every eight week regimen was recently approved by the U.S. Food and Drug Administration (FDA), based on efficacy (maintenance of vision) that was clinically equivalent at one year to the monthly ranibizumab regimen. In the second year of the studies, patients were treated with the same dose per injection as in the first year and were evaluated monthly to determine need for retreatment. Patients were treated at least every 12 weeks. All year two analyses were considered exploratory.

In an integrated analysis of the VIEW 1 and VIEW 2 studies, the visual acuity gain from baseline in the EYLEA 2mg every eight week group at week 96 was 7.6 letters compared to 8.4 letters at week 52, with an average of 11.2 injections over two years and 4.2 injections during the second year. The visual acuity gain from baseline in the monthly ranibizumab group at week 96 was 7.9 letters compared to 8.7 letters at week 52, with an average of 16.5 injections over two years and 4.7 injections during the second year. The results of each of the VIEW 1 and VIEW 2 studies were consistent with the integrated analysis.

The overall fewer average number of injections in the second year in the EYLEA 2mg every eight week group compared to the ranibizumab group (4.2 versus 4.7) was driven by the fact that fewer patients needed more intense therapy in the EYLEA group and those patients required fewer injections.

The proportion of patients who required frequent injections (six or more) during the second year was lower in the EYLEA 2mg every eight week group compared to the ranibizumab group (15.9% versus 26.5%). In the 25% of patients who required the most intense therapy (the greatest number of injections), patients in the EYLEA 2mg every eight week group required an average of 1.4 fewer injections in the second year compared to the ranibizumab group (6.6 versus 8.0). In the 25% of patients in each group who had the fewest number of injections in the second year, the average number of injections was similar (approximately 3 for both groups, corresponding to the protocol-mandated minimum number of injections).

A generally favorable safety profile was observed for both EYLEA and ranibizumab. The incidence of ocular treatment emergent adverse events was balanced across all four treatment groups in both studies, with the most frequent events associated with the injection procedure, the underlying disease and/or the aging process. The most frequent ocular adverse events (greater than 10% of patients for the overall study population) were conjunctival hemorrhage, eye pain, retinal hemorrhage, and visual acuity reduced. The most frequent serious non-ocular adverse events were typical of those reported in this elderly population who receive intravitreal treatment for wet AMD; the most frequently reported events (greater than 1% of patients for the overall study population) were falls, pneumonia, myocardial infarction and atrial fibrillation. There were no notable differences among the study arms. The incidence of arterial thrombotic events as defined by the "Anti-Platelet Trialists" group criteria was 3.2% of patients for ranibizumab and 3.3% of patients in the combined EYLEA groups.

"These second year results confirm the sustainability of the vision gains achieved by EYLEA with a less than monthly dosing frequency. Importantly, the second year data demonstrated that for patients that needed more anti-VEGF treatment, this was achieved with fewer injections using EYLEA," said George D. Yancopoulos, M.D., Ph.D., Chief Scientific Officer of Regeneron and President of Regeneron Laboratories. "As a reminder, the recommended dose for EYLEA is 2mg every eight weeks following three initial monthly injections, which demonstrated visual acuity gains that were clinically equivalent to monthly ranibizumab. Retinal physicians and their wet AMD patients consider the predictable every eight week dosing regimen for EYLEA as a significant advance that helps overcome the challenges of monthly office visits."

Further results from year two of the studies will be presented at upcoming medical conferences.

About the VIEW Program

The VIEW (VEGF Trap: Investigation of Efficacy and Safety in Wet AMD) program consists of two randomized, double-masked, Phase 3 clinical trials evaluating EYLEA in the treatment of the neovascular form of age-related macular degeneration (wet AMD). The VIEW 1 study, which randomized 1217 patients, was conducted in the United States and Canada by Regeneron. The VIEW 2 study, which randomized 1240 patients, was conducted in Europe, Asia Pacific, Japan, and Latin America by Bayer HealthCare. The study designs are essentially identical. The primary endpoint evaluation was conducted at 52 weeks.

About Wet AMD

Age-related Macular Degeneration (AMD) is a leading cause of acquired blindness. Macular degeneration is diagnosed as either dry (non-exudative) or wet (exudative). In wet AMD, new blood vessels grow beneath the retina and leak blood and fluid. This leakage causes disruption and dysfunction

of the retina creating distortion and/or blind spots in central vision, and it can account for blindness in wet AMD patients. Wet AMD is the leading cause of blindness for people over the age of 65 in the U.S. and Europe.

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IMPORTANT PRESCRIBING INFORMATION

In the United States, EYLEA is indicated for the treatment of patients with neovascular age-related macular degeneration (wet AMD).

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Please see the full Prescribing Information for EYLEA, available online at www.regeneron.com/EYLEA.fpi.pdf.

About the EYLEA™ (aflibercept) Injection Global Collaboration

Regeneron is collaborating with Bayer HealthCare on the global development of EYLEA. Bayer submitted an application for marketing authorization in Europe for wet AMD in June 2011.

Bayer HealthCare will market EYLEA outside the United States, where the companies will share equally the profits from any future sales of EYLEA. Regeneron maintains exclusive rights to EYLEA in the United States.

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About Bayer HealthCare

The Bayer Group is a global enterprise with core competencies in the fields of health care, nutrition and high-tech materials. Bayer HealthCare, a subgroup of Bayer AG with annual sales of more than EUR 16.913 billion (2010), is one of the world's leading, innovative companies in the healthcare and medical products industry and is based in Leverkusen, Germany. The company combines the global activities of the Animal Health, Consumer Care, Medical Care and Pharmaceuticals divisions. Bayer HealthCare's aim is to discover and manufacture products that will improve human and animal health worldwide. Bayer HealthCare has a global workforce of 55.700 employees and is represented in more than 100 countries. Find more information at www.bayerhealthcare.com.

Regeneron Forward-Looking Statement

This news release includes forward-looking statements that involve risks and uncertainties relating to future events and the future performance of Regeneron, and actual events or results may differ materially from these forward-looking statements. These statements concern, and these risks and uncertainties include, among others, the nature, timing, and possible success and therapeutic applications of EYLEA and Regeneron's product candidates and research and clinical programs now underway or planned, the likelihood and timing of possible regulatory approval and commercial launch of Regeneron's late-stage product candidates, determinations by regulatory and administrative governmental authorities which may delay or restrict Regeneron's ability to continue to develop or commercialize EYLEA and other products and drug candidates, competing drugs that may be superior to EYLEA and Regeneron's products and drug candidates, uncertainty of market acceptance of EYLEA and Regeneron's products and drug candidates, unanticipated expenses, the availability and cost of capital, the costs of developing, producing, and selling products, the potential for any license or collaboration agreement, including Regeneron's agreements with Sanofi and Bayer HealthCare, to be canceled or terminated without any product success, and risks associated with third party intellectual property and pending or future litigation relating thereto. A more complete description of these and other material risks can be found in Regeneron's filings with the United States Securities and Exchange Commission, including its Form 10-K for the year ended December 31, 2010 and Form 10-Q for the quarter ended September 30, 2011. Regeneron does not undertake any obligation to update publicly any forward-looking statement, whether as a result of new information, future events, or otherwise, unless required by law.

Bayer Forward-Looking Statements

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SOURCE Regeneron Pharmaceuticals, Inc.

News Provided by Acquire Media

Electronic Patent Application Fee Transmittal	
Application Number:	16397267
Filing Date:	29-Apr-2019
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-008CIPCON5
Filed as Large Entity	
Filing Fees for Utility under 35 USC 111(a)	

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

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	240	240
		Total in USD ($)		**240**

Electronic Acknowledgement Receipt	
EFS ID:	39876409
Application Number:	16397267
International Application Number:	
Confirmation Number:	8135
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-008CIPCON5
Receipt Date:	30-JUN-2020
Filing Date:	29-APR-2019
Time Stamp:	17:19:54
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$240
RAM confirmation Number	E20206TH20345313
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	0725US06_2020-06-30_Supp_IDS_trans_REGN-008CIPCON5.pdf	50733 a012973cb4cd520a7ff11fbc5358b8c1f2914360	no	2
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	0725US06__2020-06-30_Supp_IDS_SB08A_REGN-008CIPCON5.pdf	36066 2f8efdb31199c6334e6006ab21ea61bbea511801	no	2
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Non Patent Literature	BayerNews_20080928_0448_en.pdf	127802 6d6478579d4b400aca428b8b3a577d4030031a53	no	5
Warnings:					
Information:					
4	Non Patent Literature	REGN_Press_Release_Mar_27_2007.pdf	1360202 986d7edd7cdd3899ced99c0603f9540b36f32357	no	2
Warnings:					
Information:					
5	Non Patent Literature	REGN_Press_Release_May_9_2007.pdf	1348579 4aea4c46411524118a56a126ad8880d5628dc9d1	no	2
Warnings:					
Information:					
6	Non Patent Literature	REGN_Press_Release_Aug_1_2007.pdf	2839120 9d80051b30879bcf5163b4639fd18a64ca6b2bb6	no	5
Warnings:					

Information:					
7	Non Patent Literature	REGN_Press_Release_Aug_2_2007.pdf	106077 a6c28091d1985f689be1eccf605d9cbff04cb0eb	no	2
Warnings:					
Information:					
8	Non Patent Literature	REGN_Press_Release_Oct_1_2007.pdf	1457215 f6338613f2aabca358d8b8103489fbbf67826e36	no	3
Warnings:					
Information:					
9	Non Patent Literature	REGN_Press_Release_Feb_27_2008.pdf	3789068 585e6714db309793c0e0f1d5dd74666c3615a379	no	6
Warnings:					
Information:					
10	Non Patent Literature	REGN_Press_Release_Apr_28_2008.pdf	24012 21d51e0e9eb9e2b27059dd2eaf647e2220ef6395	no	2
Warnings:					
Information:					
11	Non Patent Literature	REGN_Press_Release_Aug_19_2008.pdf	23293 5d13932b21799c081aff58b6fa4fc0a8e6b4b825	no	2
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12	Non Patent Literature	REGN_Press_Release_Feb_26_2009.pdf	3449787 c5f9143255909 8f3ad1fb2789fde8c0fd873e91e	no	5
Warnings:					
Information:					
13	Non Patent Literature	REGN_Press_Release_Apr_30_2009.pdf	107443 9b7019f9ac83ae628b74e4cf5d36232c548ea7e9	no	2
Warnings:					
Information:					

14	Non Patent Literature	REGN_Press_Release_Jul_23_2009.pdf	1070186 5d38c7318355bbb4bd99263a4d5c178ce9c0eb36	no	2
Warnings:					
Information:					
15	Non Patent Literature	REGN_Press_Release_Nov_19_2010.pdf	350695 b241755a11b1083bf843f27bbf27849ece1d60a6	no	1
Warnings:					
Information:					
16	Non Patent Literature	REGN_Press_Release_Jan_18_2011.pdf	1765835 834d1807fb38ebe24b62e6859d6c78251db8b218	no	3
Warnings:					
Information:					
17	Non Patent Literature	REGN_Press_Release_Feb_9_2011.pdf	1371797 aab4ca6426678222de6ccf81389495a08d533ce5	no	2
Warnings:					
Information:					
18	Non Patent Literature	REGN_Press_Release_Feb_22_2011.pdf	110830 1c15ab6a0cda244ad867d79f5839abc72511f8963	no	6
Warnings:					
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19	Non Patent Literature	REGN_Press_Release_Apr_8_2011.pdf	1480611 a13f642ce8b29401bd55e0d61db1de29d171f413	no	2
Warnings:					
Information:					
20	Non Patent Literature	REGN_Press_Release_Apr_18_2011.pdf	17501 a824d1d3a227ce42763b0da65601593f396c765b	no	2
Warnings:					
Information:					

21	Non Patent Literature	REGN_Press_Release_Jun_7_2011.pdf	1463628 41e6c89cfa55d29dff0736ab0e82cebfbec32dea	no	2
Warnings:					
Information:					
22	Non Patent Literature	REGN_Press_Release_Jun_17_2011.pdf	19498 afef5a7b39b651100449db5b31c908297f673434	no	2
Warnings:					
Information:					
23	Non Patent Literature	REGN_Press_Release_Aug_17_2011.pdf	581399 8e96e29aa8c022ae65c25ab110c11f914459 3e27	no	2
Warnings:					
Information:					
24	Non Patent Literature	REGN_Press_Release_Nov_18_2011.pdf	19527 8864153c02303fe0f6969e05c4cf59b74582f 9f3	no	2
Warnings:					
Information:					
25	Non Patent Literature	REGN_Press_Release_Nov_28_2011.pdf	27840 42379aa75f28abacc9ffca7fea7a138a3fcd37 4f	no	3
Warnings:					
Information:					
26	Non Patent Literature	REGN_Press_Release_Dec_5_2011.pdf	32377 cc45cb0792fb752a70ed96149f7f07e1638a d13f	no	3
Warnings:					
Information:					
27	Fee Worksheet (SB06)	fee-info.pdf	31040 0ea746dc11ec4ecd51485028a54064743 7dc4adf	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	23062161		

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-008CIPCON5
	Confirmation No.	8135
	First Named Inventor	George D. Yancopoulos
	Application Number	16/397,267
	Filing Date	April 29, 2019
	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-008CIPCON5.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 30 June 2020

By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	16/397,267
				Filing Date	April 29, 2019
				First Named Inventor	George D. Yancopoulos
				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	1	of	5	Attorney Docket Number	REGN-008CIPCON5

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	

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				Art Unit	1647
				Examiner Name	Jon McClelland Lockard
Sheet	2	of	5	Attorney Docket Number	REGN-008CIPCON5

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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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Sheet	3	of	5	Attorney Docket Number	REGN-008CIPCON5

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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)	
	40	Regeneron SEC Form 10-Q (July 28, 2011)	
	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	
	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)	

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

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International Bureau

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10/609,775 30 June 2003 (30.06.2003) US

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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.

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[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

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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

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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

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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

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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,

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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

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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

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(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

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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.

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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®;

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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,

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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

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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.

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[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

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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

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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

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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

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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

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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}

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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.

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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.

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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.

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145                 150                 155                 160
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
                165                 170                 175
Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
            180                 185                 190
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
        195                 200                 205
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
    210                 215                 220
Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Cys Pro Pro Cys
225                 230                 235                 240
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
                245                 250                 255
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
            260                 265                 270
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
        275                 280                 285
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
    290                 295                 300
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
305                 310                 315                 320
```


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```
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
                325                 330                 335
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
            340                 345                 350
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
        355                 360                 365
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
    370                 375                 380
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
385                 390                 395                 400
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
                405                 410                 415
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
            420                 425                 430
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
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Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455
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<213> homo sapiens

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<210> 12
<211> 1444
<212> DNA
<213> homo sapiens

<400> 12

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tgcttctcac aggatctagt tccggaggta gacctttcgt agagatgtac agtgaaatcc 180
ccgaaattat acacatgact gaaggaaggg agctcgtcat tccctgccgg gttacgtcac 240
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gcataatctg ggacagtaga aagggcttca tcatatcaaa tgcaacgtac aaagaaatag 360
ggcttctgac ctgtgaagca acagtcaatg ggcatttgta taagacaaac tatctcacac 420
atcgacaaac caatacaatc atagatatcc agctgttgcc caggaagtcg ctggagctgc 480
tggtagggga gaagctggtc ctcaactgca ccgtgtgggc tgagtttaac tcaggtgtca 540
cctttgactg ggactaccca gggaagcagg cagagcgggg taagtgggtg cccgagcgac 600
gctcccaaca gacccacaca gaactctcca gcatcctgac catccacaac gtcagccagc 660
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ccgaggtcat tgtgcatgaa aatggcccgg gcgacaaaac tcacacatgc ccaccgtgcc 780
cagcacctga actcctgggg ggaccgtcag tcttcctctt ccccccaaaa cccaaggaca 840
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accctgaggt caagttcaac tggtacgtgg acggcgtgga ggtgcataat gccaagacaa 960
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ccctgccccc atcccgggat gagctgacca agaaccaggt cagcctgacc tgcctggtca 1200
aaggcttcta tcccagcgac atcgccgtgg agtgggagag caatgggcag ccggagaaca 1260
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actacaagac cacgcctccc gtgctggact ccgacggctc cttcttcctc tatagcaagc 1320
tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg 1380
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ccgc 1444

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<212> PRT
<213> homo sapiens

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35 40 45
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu
50 55 60
Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
65 70 75 80
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu
85 90 95
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys
100 105 110
Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Ile Gln
115 120 125
Leu Leu Pro Arg Lys Ser Leu Glu Leu Leu Val Gly Glu Lys Leu Val
130 135 140
Leu Asn Cys Thr Val Trp Ala Glu Phe Asn Ser Gly Val Thr Phe Asp
145 150 155 160
Trp Asp Tyr Pro Gly Lys Gln Ala Glu Arg Gly Lys Trp Val Pro Glu
165 170 175
Arg Arg Ser Gln Gln Thr His Thr Glu Leu Ser Ser Ile Leu Thr Ile
180 185 190
His Asn Val Ser Gln His Asp Leu Gly Ser Tyr Val Cys Lys Ala Asn
195 200 205
Asn Gly Ile Gln Arg Phe Arg Glu Ser Thr Glu Val Ile Val His Glu
210 215 220
Asn Gly Pro Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys

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385 390 395 400
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445
Leu Ser Leu Ser Pro Gly Lys
450 455

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<213> homo sapiens

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<210> 15
<211> 28
<212> DNA
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<400> 15
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<212> DNA
<213> homo sapiens

<400> 17
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<212> DNA
<213> homo sapiens

<400> 18
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<211> 48
<212> DNA
<213> homo sapiens

<400> 19
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<211> 57
<212> DNA

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<213> homo sapiens

<400> 20
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<211> 39
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<213> homo sapiens

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<212> DNA
<213> homo sapiens

<400> 22
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<210> 23
<211> 235
<212> PRT
<213> homo sapiens

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Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu
35 40 45
Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr
50 55 60
Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys
65 70 75 80
Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
85 90 95
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
100 105 110
Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
115 120 125
Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
130 135 140
Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
145 150 155 160
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
165 170 175
Val Asn Thr Gln Ser Gly Ser Glu Met Lys Arg Asp Leu Lys Lys Phe
180 185 190
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
195 200 205
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
210 215 220
Phe Val Arg Val His Glu Lys Gly Pro Gly Cys
225 230 235

<210> 24
<211> 435

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<212> PRT
<213> homo sapiens

<400> 24

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Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu
        35                  40                  45
Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr
    50                  55                  60
Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys
65                  70                  75                  80
Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
                85                  90                  95
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
            100                 105                 110
Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
        115                 120                 125
Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
    130                 135                 140
Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
145                 150                 155                 160
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
                165                 170                 175
Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
            180                 185                 190
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
        195                 200                 205
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
    210                 215                 220
Phe Val Arg Val His Glu Lys Gly Pro Gly Arg Pro Phe Val Glu Met
225                 230                 235                 240
Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu
                245                 250                 255
Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys
            260                 265                 270
Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp
        275                 280                 285
Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile
    290                 295                 300
Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr
305                 310                 315                 320
Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val Leu
                325                 330                 335
Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val Leu
            340                 345                 350
Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn Trp
        355                 360                 365
Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg Asp
    370                 375                 380
Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr Leu
385                 390                 395                 400
Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys Ala
                405                 410                 415
Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg Val
            420                 425                 430
His Glu Lys
        435
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<210> 25
<211> 238
<212> PRT
<213> homo sapiens

<400> 25
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20 25 30
Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu
35 40 45
Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr
50 55 60
Val Thr Leu Lys Lys Phe Pro Leu Asn Thr Leu Ile Pro Asn Gly Lys
65 70 75 80
Ala Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
85 90 95
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
100 105 110
Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
115 120 125
Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
130 135 140
Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
145 150 155 160
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
165 170 175
Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
180 185 190
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
195 200 205
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
210 215 220
Phe Val Arg Val His Glu Lys Gly Pro Gly Ala Cys Gly Cys
225 230 235

<210> 26
<211> 240
<212> PRT
<213> homo sapiens

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20 25 30
Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu
35 40 45
Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr
50 55 60
Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys
65 70 75 80
Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
85 90 95
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
100 105 110
Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
115 120 125

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Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
    130                 135                 140
Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
145                 150                 155                 160
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
                165                 170                 175
Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
            180                 185                 190
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
        195                 200                 205
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
    210                 215                 220
Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Cys Pro Pro Cys
225                 230                 235                 240

<210> 27
<211> 240
<212> PRT
<213> homo sapiens

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            20                  25                  30
Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu
        35                  40                  45
Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr
    50                  55                  60
Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys
65                  70                  75                  80
Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
                85                  90                  95
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
            100                 105                 110
Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
        115                 120                 125
Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
    130                 135                 140
Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
145                 150                 155                 160
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
                165                 170                 175
Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
            180                 185                 190
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
        195                 200                 205
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
    210                 215                 220
Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Ser Pro Pro Cys
225                 230                 235                 240

<210> 28
<211> 237
<212> PRT
<213> homo sapiens

<400> 28
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
 1               5                  10                  15
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```
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Asp Thr Gly Arg Pro
            20                  25                  30
Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu
        35                  40                  45
Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr
    50                  55                  60
Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys
65                  70                  75                  80
Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
                85                  90                  95
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
            100                 105                 110
Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
        115                 120                 125
Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
    130                 135                 140
Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
145                 150                 155                 160
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
                165                 170                 175
Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
            180                 185                 190
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
        195                 200                 205
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
    210                 215                 220
Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Cys
225                 230                 235
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<210> 29
<211> 434
<212> PRT
<213> homo sapiens

<400> 29

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


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```
     195                 200                 205
Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly
    210                 215                 220
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
225                 230                 235                 240
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu
                245                 250                 255
Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
            260                 265                 270
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg
        275                 280                 285
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
    290                 295                 300
Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu
305                 310                 315                 320
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
                325                 330                 335
Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu
            340                 345                 350
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
        355                 360                 365
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
    370                 375                 380
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp
385                 390                 395                 400
Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His
                405                 410                 415
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu
            420                 425                 430
Gly Lys
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(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

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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

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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

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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,

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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).

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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] Results. 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

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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 $CD31^{+++}$/$LYVE\text{-}1^{-}$ blood vessels were regularly accompanied by $LYVE\text{-}1^{+++}$/$CD31^{+}$ 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

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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.

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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.

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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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The Schepens Eye Research Institute

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FREE
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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

M. S. Benz; Q. D. Nguyen; K. Chu; A. Cahn; I. Grimes; A. Ingerman; J. M. Cedarbaum

+ Author Affiliations & Notes

Investigative Ophthalmology & Visual Science May 2007, Vol.48, 4549. doi:

Abstract

Purpose:: To determine the safety, tolerability, and biological effect of repeated intravitreal (ITV) injection of VEGF Trap in patients with neovascular age-related macular degeneration (AMD).

Methods:: Five groups of patients with neovascular AMD were randomized in a balanced ratio to receive a series of ITV injections of one of 3 dose levels of VEGF Trap (0.5, 2 or 4 mg) into the study eye at 4- or 12-week intervals over a 12-week period. Measures of bioactivity included changes from baseline in best-corrected ETDRS visual acuity (BCVA), foveal thickness, and macular volume determined by optical coherence tomography, as well as total lesion and CNV area determined by fluorescein angiography. Dosing was continued beyond week 12 using a criteria-based schedule.

Results:: The study is currently ongoing and treatment assignments remain masked. No ocular serious adverse events, no identifiable intraocular inflammation or serious drug-related systemic adverse events have been reported to date.

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Conclusions:: Repeated intravitreal VEGF Trap administration according to the dosing regimens employed in this study appears to be safe and well tolerated. Safety and bioeffect data will be updated at time of presentation.

Clinical Trial:: www.clinicaltrials.gov NCT00320788

Keywords: age-related macular degeneration • retina • clinical (human) or epidemiologic studies: outcomes/complications

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ARVO Annual Meeting Abstract | May 2007

Results of a Phase I Study of Intravitreal VEGF Trap in Subjects With Diabetic Macular Edema: The CLEAR-IT DME Study

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Investigative Ophthalmology & Visual Science May 2007, Vol.48, 1430. doi:

Abstract

Purpose:: To determine the safety, tolerability, and bioactivity of a single dose (4.0mg) of intravitreal VEGF Trap in patients with diabetic macular edema (DME).

Methods:: Five patients with DME, foveal thickness ≥ 250µm measured by optical coherence tomography (OCT) and ETDRS best-corrected visual acuity (BCVA) of ≤ 20/40 and ≥ 20/320 were administered a single intravitreal injection of 4 mg VEGF Trap at day 0. Safety assessments included eye examinations, vital signs, and laboratory tests. Measures of bioactivity included changes from baseline in BCVA, centerpoint retinal thickness (CRT), and leakage on fluorescein angiography. Subjects were monitored for 6 weeks following VEGF Trap administration.

Results:: Mean patient age was 65.2 years (range=56-75); 4 were Type 2 diabetics. Mean duration of diabetes prior to treatment was 26 years. All had received prior treatment for DME. No severe ocular or serious systemic adverse events related to study drug were noted. Mean baseline BCVA was 69 letters and mean baseline CRT was 407µm. Four patients had improvements in BCVA, ranging from 6 to 10 letters at 4 weeks post-injection. The mean decrease in centerpoint retinal thickness was 115µm at 4 weeks.

Conclusions:: A single intravitreal dose of VEGF Trap was well-tolerated and led to a reduction in CRT and an improvement in BCVA. Although the number of subjects is small, preliminary evidence for bioactivity of VEGF Trap in patients with DME was detected.

Additional studies are being planned to identify the potential therapeutic role of intravitreal VEGF Trap in DME.

Clinical Trial:: www.clinicaltrials.gov NCT 00320814

Keywords: diabetic retinopathy • macula/fovea • clinical (human) or epidemiologic studies: treatment/prevention assessment/controlled clinical trials

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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

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Investigative Ophthalmology & Visual Science April 2009, Vol.50, 1887. doi:

Abstract

Purpose: : To examine the effects of VEGF Trap-Eye on patient-reported vision-specific Quality of Life (QoL) using the National Eye Institute Visual Function Questionnaire (NEI VFQ-25) in CLEAR-IT 2: a Phase 2 randomized trial in patients with subfoveal choroidal neovascularization (CNV) due to AMD.

Methods: : CLEAR-IT 2 was a randomized, double masked, multi-center Phase 2 trial. Patients received intravitreal injections of VEGF Trap-Eye 0.5 or 2.0 mg monthly or 0.5, 2.0, or 4.0 mg quarterly for 12 weeks, followed by PRN dosing based on results of OCT and clinical examination out to 52 weeks. The NEI-VFQ 25 was administered to patients at baseline, week 12, and week 52. QoL was assessed at 52 weeks by change in mean score from baseline. The NEI-VFQ 25 subscales are scored from 0-100; a positive difference represents improved functioning or reduced dependency. Pre-specified subscales included near activities, distance activities, and vision related dependency.

Results: : The mean overall change from baseline to 52 weeks in the total score of the NEI-VFQ 25 for all treatment groups combined (n=145) was +4.5. For all groups combined patients had mean changes of +5.7 for near activities, +3.4 for distance activities and +5.8 for vision related dependency. Patients receiving the 2.0 mg dose monthly for the first 12 weeks (2mg q4 group, n=28) had a change from baseline of +4.5 in the total score, +6.8 for near activities, +4.6 for distance activities, and +11.6 for vision related dependency at 52 weeks.

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Conclusions: : Patients treated with multiple doses of VEGF Trap-Eye over a 52 week period had overall improvements in the patient-reported vision-specific QoL (total scores as well as subscales) as assessed by the NEI-VFQ 25. As a clinically meaningful change is often considered to be ±5 points, the most notable improvements were seen in the 2mg q4 group for near activities and for vision related dependency.

Clinical Trial: : www.clinicaltrials.gov NCT00320788

Keywords: age-related macular degeneration • quality of life • clinical (human) or epidemiologic studies: treatment/prevention assessment/controlled clinical trials

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VEGF Trap-Eye In CRVO: Primary Endpoint Results Of The Phase 3 COPERNICUS Study

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Investigative Ophthalmology & Visual Science April 2011, Vol.52, 6643. doi:

Abstract

Purpose: : VEGF Trap-Eye is an intravitreally administered fusion protein that is designed to bind the pro-angiogenic factors VEGF-A and placental growth factor with higher affinity than their natural receptors. This study evaluated the efficacy and safety of VEGF Trap-Eye in patients with macular edema secondary to central retinal vein occlusion (CRVO) after 24 weeks of treatment.

Methods: : In this randomized, double-masked, controlled Phase 3 study, 114 patients received 6 monthly injections of 2 mg VEGF Trap-Eye and 73 patients received control sham injections. Visual acuity was measured as a score based on the total number of Early Treatment of Diabetic Retinopathy Study (ETDRS) letters read correctly. The primary endpoint was the proportion of patients who gained at least 15 ETDRS letters from baseline at 24 weeks. A key secondary endpoint was the mean change in best-corrected visual acuity from baseline at 24 weeks.

Results: : The primary endpoint was met in this study: 56.1% of patients receiving 2 mg VEGF Trap-Eye monthly gained at least 15 letters of vision from baseline, compared with 12.3% of patients receiving sham injections ($p<0.0001$). Patients receiving VEGF Trap-Eye gained a mean of 17.3 letters of vision compared with a mean loss of 4.0 letters with sham injection ($p<0.001$). The most common adverse events were those typically associated with intravitreal injections and/or the underlying disease. The proportions of patients who experienced serious ocular adverse events were 3.5% in the VEGF Trap-Eye group and 13.5% in the sham group. The incidence of non-ocular serious adverse events was generally well balanced between the treatment and sham groups.

Conclusions: : Dosing monthly with 2 mg VEGF Trap-Eye in patients with macular edema secondary to CRVO resulted in a statistically significant improvement in visual acuity compared with control sham treatment. VEGF Trap-Eye was generally well tolerated and had a generally favorable safety profile.

Clinical Trial: : http://www.clinicaltrials.gov NCT00943072

Keywords: vascular endothelial growth factor • visual acuity • vascular occlusion/vascular occlusive disease

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CLEAR-IT 2: Phase 2, Randomized, Controlled Dose-and Interval-Ranging Study of Intravitreal VEGF Trap Eye in Patients With Neovascular Age-Related Macular Degeneration: Predictive Factors for Visual Acuity Outcome at One Year

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Investigative Ophthalmology & Visual Science April 2009, Vol.50, 1255. doi:

Abstract

Purpose: : To evaluate potential predictive factors for visual acuity outcomes in patients with neovascular AMD after repeated intravitreal injections of VEGF Trap-Eye over one year.

Methods: : CLEAR-IT 2 was a double-masked, multi-center Phase 2 trial in AMD patients randomized to receive VEGF Trap-Eye 0.5 or 2.0 mg monthly or 0.5, 2.0, or 4.0 mg quarterly (at baseline and week 12) with monthly reassessment and PRN dosing to 1 year. Subgroups of patients were identified based on age, baseline best-corrected visual acuity (BCVA), baseline lesion size, and previous treatment for neovascular AMD.

Results: : Data through one year for all dose groups combined (n=157) demonstrated significant improvement in BCVA (mean increase of 5.3 letters, $p<0.0001$.) Analyses of the subgroups identified above generated the following results for all treatment groups combined: Patients that were ≤75 years old (n=53) gained an average of 8.26 (±13.55) letters as opposed to those >75 years old (n=104) who gained 3.73 (±13.31) letters ($p=0.046$). Those with a BCVA ≤54 letters at baseline (n=65) gained an average of 7.54 (±15.44) letters as opposed to those with a BCVA at baseline > 54 letters (n=92) who gained

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3.65 (±11.8) letters (p=0.076). Those who began the study with a lesion size ≤4DA (n=124) gained an average of 5.52 (±13.63) letters, while those who began the study with lesions >4DA gained 4.27 (±13.23) letters (p=0.63). Patients who were treatment naïve at the start of the study (n=137) gained an average of 4.8 (±13.7) letters, while those who received previous treatment (n=20) gained an average of 8.4 (±12.09) letters (p=0.27).

Conclusions: : In this study, ≤75 years of age at baseline predicted greater visual acuity gains following multiple treatments of VEGF Trap-Eye. In addition, worse baseline visual acuity was suggestive of better visual acuity outcomes. Baseline lesion size and previous treatment status did not significantly affect treatment responses.

Clinical Trial: : www.clinicaltrials.gov NCT00320788

Keywords: age-related macular degeneration • choroid: neovascularization • vascular endothelial growth factor

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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

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Objective: To evaluate anatomic outcomes and vision, injection frequency, and safety during the as-needed (PRN) treatment phase of a study evaluating a 12-week fixed dosing period followed by PRN dosing to week 52 with vascular endothelial growth factor (VEGF) Trap-Eye for neovascular (wet) age-related macular degeneration (AMD).

Design: Multicenter, randomized, double-masked trial.

Participants: We included 159 patients with subfoveal choroidal neovascularization (CNV) secondary to wet AMD.

Methods: Patients were randomly assigned to 1 of 5 intravitreal VEGF Trap-Eye treatment groups: 0.5 mg or 2 mg every 4 weeks or 0.5, 2, or 4 mg every 12 weeks during the fixed-dosing period (weeks 1–12). From weeks 16 to 52, patients were evaluated monthly and were retreated PRN with their assigned dose (0.5, 2, or 4 mg).

Main Outcome Measures: Change in central retinal/lesion thickness (CR/LT), change in total lesion and CNV size, mean change in best-corrected visual acuity (BCVA), proportion of patients with 15-letter loss or gain, time to first PRN injection, reinjection frequency, and safety at week 52.

Results: The decrease in CR/LT at week 12 versus baseline remained significant at weeks 12 to 52 (−130 μm from baseline at week 52) and CNV size regressed from baseline by 2.21 mm^2 at 48 weeks. After achieving a significant improvement in BCVA during the 12-week, fixed-dosing phase for all groups combined, PRN dosing for 40 weeks maintained improvements in BCVA to 52 weeks (5.3-letter gain; $P<0.0001$). The most robust improvements and consistent maintenance of visual acuity generally occurred in patients initially dosed with 2 mg every 4 weeks for 12 weeks, demonstrating a gain of 9 letters at 52 weeks. Overall, a mean of 2 injections was administered after the 12-week fixed-dosing phase, and the mean time to first reinjection was 129 days; 19% of patients received no injections and 45% received 1 or 2 injections. Treatment with VEGF Trap-Eye was generally safe and well tolerated, with few ocular or systemic adverse events.

Conclusions: PRN dosing with VEGF Trap-Eye at weeks 16-52 maintained the significant anatomic and vision improvements established during the 12-week fixed-dosing phase with a low frequency of reinjections. Repeated dosing with VEGF Trap-Eye was well tolerated over 52 weeks of treatment.

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Vascular endothelial growth factor (VEGF) is a critical regulator of normal ocular vasculogenesis and angiogenesis during development.[1–3] Vascular endothelial growth factor also plays a central role in the abnormal growth of new blood vessels in the retina, as well as in vascular leakage that causes retinal edema and thickening, both of which characterize diseases such as neovascular (wet) age-related macular degeneration (AMD) and diabetic retinopathy that lead to loss of retinal function.[3–6] Of the various members of the VEGF gene family, VEGF-A and placental growth factor (PlGF) are the factors implicated in pathologic angiogenesis and the pathogenesis of AMD (Invest Ophthalmol Vis Sci 50 [Suppl]: 2943,2009).[7–9]

An improved understanding of the pivotal role of VEGF in pathologic angiogenesis has resulted in the development and use of intravitreal anti-VEGF therapies in wet AMD and other eye diseases that have an angiogenesis-based etiology.[10–12] Pegaptanib, an oligoribonucleotide aptamer,

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and ranibizumab, a humanized monoclonal antibody fragment, are anti-VEGF therapies currently available for intravitreal treatment of neovascular AMD. In pivotal trials, pegaptanib mainly slowed loss of visual acuity, whereas ranibizumab improved visual function in a substantial proportion of patients.[13–16] Bevacizumab is an off-label intravitreal anti-VEGF therapy that has also been shown in less rigorous studies to improve visual function.[17–20]

The beneficial results with ranibizumab were obtained with a fixed-dosing regimen requiring an injection of ranibizumab 0.5 or 0.3 mg every month for 2 years in the pivotal phase 3 studies, Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular Age-Related Macular Degeneration (MARINA) and Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in Age-Related Macular Degeneration (ANCHOR).[13,15] Several studies were undertaken to examine different dosing regimens for ranibizumab. In a large, randomized study in which patients were initially treated with a 3-month loading regimen of ranibizumab and then dosed at regular quarterly intervals, the initial gains after the loading regimen were not maintained at a year.[21] A 40-patient uncontrolled, open-label, single-site, Prospective Optical Coherence Tomography Imaging of Patients Treated with intra-Ocular ranibizumab (PrONTO) trial evaluated an as-needed (PRN) dosing regimen (based on monthly evaluation of changes in retinal thickness and edema using optical coherence tomography [OCT]) after 3 consecutive monthly injections. Visual acuity outcomes at 12 and 24 months were comparable with those observed in the pivotal phase 3 studies and were attained with fewer intravitreal injections.[22,23] However, in a larger randomized study, the gains in visual acuity after an initial 3-month loading regimen of ranibizumab were not maintained with subsequent protocol-defined retreatment.[24]

Vascular endothelial growth factor Trap-Eye (VEGF Trap-Eye) is a potent, specific VEGF antagonist that binds and inactivates circulating VEGF and VEGF in the extravascular space. It was developed specifically as an ultrapurified, isoosmotic solution for ophthalmic use.[25] Consisting of extracellular portions of VEGF receptors 1 and 2 fused to the Fc portion of human immunoglobulin G, VEGF Trap-Eye binds both VEGF-A or PlGF and forms an inert 1:1 complex with the growth factor dimers.[24,25] Thus, VEGF Trap-Eye has broader anti-VEGF activity compared with pegaptanib, which binds only the VEGF-A_{165} isoform,[26] and ranibizumab, which neutralizes all active isoforms of VEGF-A, but not PlGF.[27] Because VEGF Trap-Eye contains only human sequences, its potential for immunogenicity is low. A key differentiating feature of VEGF Trap-Eye is its picomolar affinity for VEGF ligands, which is substantially higher than that of the natural receptors or anti-VEGF monoclonal antibodies.[25,28,29] The clinical relevance of the higher binding affinity of VEGF Trap-Eye remains unknown, but it is thought that it might lead to more persistent VEGF blockade and a theoretically longer dosing interval between injections to maintain visual acuity relative to currently available anti-VEGF treatments.[30]

The clinical efficacy of VEGF-Trap Eye was initially demonstrated in the CLinical Evaluation of Anti-angiogenesis in the Retina Intravitreal Trial (CLEAR-IT 1), a 6-week phase 1 sequential cohort, single-ascending-dose (0.05 to 4 mg) study of intravitreal VEGF Trap-Eye in patients with neovascular AMD.[31] The efficacy and safety of repeated dosing with VEGF Trap-Eye were subsequently examined in the phase 2 CLEAR-IT 2 study, which consisted of an initial 12-week fixed dosing period with 1 of 5 monthly or quarterly regimens of VEGF Trap-Eye, followed by PRN dosing from weeks 16 to 52. Detailed results to 16 weeks for the fixed-dosing phase, including the primary endpoint at week 12, are presented in the accompanying article (Brown et al., in this issue, pp 1089-97). At 12 weeks, treatment with VEGF Trap-Eye resulted in a significant reduction in central retinal/lesion thickness (CR/LT) of -119 μm and a significant improvement in mean BCVA of 5.7 letters for all groups combined, and gains of >8 letters in the monthly dosing groups. The finding that improvements in visual acuity and retinal thickness were greater in the monthly dosing groups than in the quarterly dosing groups at week 12, support the need for an initial intensive monthly loading dose phase. Patients were treated with a PRN dosing regimen through week 52 to explore whether the high affinity of VEGF Trap-Eye for VEGF-A and PlGF could translate into the maintenance of initial visual acuity gains through 1 year with less frequent intravitreal injections. Results of the continued dosing phase of the CLEAR-IT 2 study are reported herein.

Materials and Methods

Study Design

The primary objectives of the study were to assess the effect of intravitreal VEGF Trap-Eye on CR/LT and to assess the ocular and systemic safety and tolerability of repeated doses of VEGF Trap-Eye in patients with choroidal neovascularization (CNV) associated with wet AMD. A key secondary objective was to assess the effect of VEGF Trap-Eye on BCVA.

This study was a double-masked, prospective, randomized, dose- and interval-ranging study in which 5 groups of approximately 30 patients each were assigned to a fixed-dose of intravitreal VEGF Trap-Eye in the study eye during the first 12 weeks of dosing, followed by PRN dosing from weeks 16 to 52 (Fig 1, available online at http://aaojournals.org). The VEGF Trap-Eye regimens were 0.5 mg or 2 mg at 4-week intervals (0.5q4 or 2q4 on day 1 and at weeks 4, 8, and 12 for a total of 4 treatments) or 0.5, 2, or 4 mg every 12 weeks (0.5q12, 2q12, or 4q12 on day 1 and week 12 for a total of 2 treatments). During the PRN dosing phase beginning at week 16, patients received the same dose of VEGF Trap-Eye (0.5, 2, or 4 mg) as received during the fixed-dosing phase (Fig 2).

The study protocol was approved by the ethics committee at every institution and was conducted according to the recommendations of Good Clinical Practice and the Declaration of Helsinki. The study was compliant with the rules and regulations under the Health Insurance Portability and Accountability Act of 1996. All patients provided written informed consent to participate in the study. The CLEAR-IT 2 study is registered with ClinicalTrials.gov (NCT00320788).

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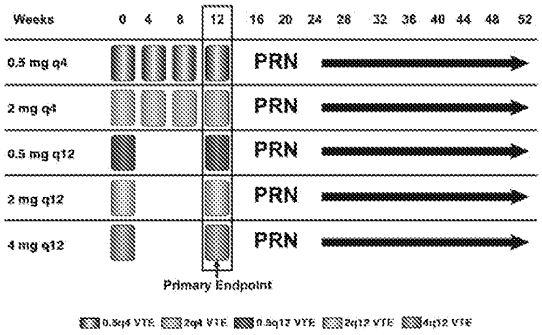


Figure 2. Study schedule. During the 12-week fixed dosing phase, patients in the monthly dosing groups received 0.5 or 2 mg of VEGF Trap-Eye every 4 weeks on day 0 and at weeks 4, 8, and 12 for a total of 4 doses; those in the quarterly dosing groups received 0.5, 2, or 4 mg of VEGF Trap-Eye every 12 weeks on day 0 and at week 12 for a total of 2 doses. Beginning at week 16 and continuing to week 52, patients were assessed every 4 weeks and, if needed, were retreated with the same dose of VEGF Trap-Eye as in the fixed dosing phase. The primary study endpoint was assessed at week 12. q = every; PRN = as-needed; VEGF = vascular endothelial growth factor; VTE = VEGF Trap-Eye; 0.5q4 = 0.5 mg every 4 weeks; 2q4 = 2 mg every 4 weeks; 0.5q12 = 0.5 mg every 12 weeks; 2q12 = 2 mg every 12 weeks; 4q12 = 4 mg every 12 weeks.

Patient Population

The study enrolled patients >50 years old who had a diagnosis of subfoveal CNV secondary to wet AMD and central retinal thickness ≥300 μm, Early Treatment of Diabetic Retinopathy Study (ETDRS) BCVA of 73 to 34 letters, loss of ≥5 ETDRS letters in BCVA over the preceding 6 months for previously treated patients with minimally classic or occult lesions, linear diameter of lesion ≤5400 μm by fluorescein angiography (FA), subretinal hemorrhage sparing the fovea and comprising ≤50% of total lesion, area of scar ≤25% of total lesion, and sufficient clarity of ocular media to allow retinal photography.

Key exclusion criteria were history of vitreous hemorrhage in preceding 4 weeks; aphakia or pseudophakia with absence of a posterior capsule (unless as a result of a yttrium aluminum garnet capsulotomy); significant subfoveal atrophy or scarring; presence of other causes of CNV in either eye; previous treatments for AMD in the study eye within 12 weeks for photodynamic therapy, 8 weeks for pegaptanib sodium, or 24 weeks for intravitreal or juxtascleral steroids; no other treatments for AMD (thermal laser, surgery, or intraocular/systemic anti-VEGF therapy) were allowed; any retinal vascular disease other than CNV in either eye; active ocular inflammation or infection; history of trabeculectomy or pars plana vitrectomy; history of myocardial infarction, stroke, transient ischemic attack, symptomatic peripheral vascular disease, or treatment for congestive heart failure within the last 6 months; and other conditions or laboratory abnormalities that might interfere with patient participation in the study.

Retreatment Criteria

During the fixed-dosing phase, 1 eye was designated as the study eye and all evaluations were conducted on that eye as described previously (Brown et al, in this issue, pp 1089-97). Beginning at week 16, patients were evaluated every 4 weeks to determine the need for continued dosing. After week 16, the study eye was reinjected with VEGF Trap-Eye if any of the following changes were observed by the evaluating practitioner: An increase in CR/LT ≥100 μm as measured by OCT; a loss of ≥5 ETDRS letters in conjunction with recurrent fluid as indicated by OCT; persistent fluid as indicated by OCT; new-onset classic neovascularization; new or persistent leak on FA; or new macular hemorrhage.

Endpoints and Assessments

Assessments were performed at scheduled clinic visits on days 1 and 7, at week 4, and every 4 weeks thereafter to week 52. At each visit, patients underwent a full ophthalmologic examination, including visual acuity testing, indirect ophthalmoscopy and slit lamp examination, intraocular pressure (IOP) measurement, and OCT. Fundus photography and FA were performed at baseline and at weeks 4, 12, 24, 36, and 48.

The primary efficacy endpoint was reduction of CR/LT from baseline to 12 weeks, at the end of the fixed-dosing phase. The variables assessed during the PRN dosing phase included change from baseline in CR/LT and mean change from baseline in CNV size determined by FA at 48 weeks, the last mandatory FA in the study; BCVA at 52 weeks; proportions of patients with avoidance of moderate vision loss (loss of <15 letters); stabilization or improvement in visual acuity (gain of ≥0 letters); and significant vision gain (gain of ≥15 letters) at 52 weeks; time to first reinjection after week 12; and mean number of injections over the PRN period.

The CR/LT was determined from Stratus OCT (Version 4.0 or higher; CarlZeiss, Jena, Germany) scans read at a masked independent central reading center (Digital OCT Reading Center, Cleveland, OH). The CR/LT was defined as the distance between the inner limiting membrane and posterior border of retinal pigment epithelial/choriocapillaris complex including any subretinal fluid and thickness of any observable choroidal neovascular membrane or scar tissue in central 1 mm of posterior pole scan.

Changes in the size of the total lesion and the CNV component were evaluated with FA. The CNV size was defined as the area of visible CNV (classic or occult) with angiographic evidence of late leakage or pooling of dye. Angiographic images were transmitted to the masked reading center for review (Digital Angiography Reading Center, New York, NY). At least 1 designated photographer was certified by the Reading Center before enrollment of the first patient at each site.

Certified examiners assessed BCVA by using the ETDRS protocol at 4 m. Examiners were masked to treatment assignment, and performed no other study assessments.

Safety assessments included IOP (measured preinjection and approximately 30 minutes postinjection), ophthalmologic examinations for ocular toxicity, adverse events (AEs), serious AEs (SAEs), clinical laboratory tests, and vital signs.

Statistical Analysis

Efficacy assessments were made on the full analysis set, which included all patients who received study treatment and had a baseline and ≥1 postbaseline assessment. Safety assessments were performed on all patients who received study treatment.

The primary analysis was a paired comparison t test of the change in CR/LT from baseline to week 12 for all groups combined. If this was significant, an analysis of covariance was done on the 5 individual groups. A similar analysis was done for all continuous measures at all time points. Missing values were imputed using the last-observation-carried-forward method for continuous measures. The durability of the effect was assessed by evaluating all of the endpoints out to week 52. All of the analyses shown below were done using the same methods at week 12 and week 52 (week 48 for FA parameters).

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Table 2. Baseline Demographic and Clinical Characteristics

Characteristic	All Treated Patients (n = 157)
Age, y (mean [range])	78.3 (53–94)
Gender (%M:%F)	38:62
Disease duration, months (mean [range])	3.9 (0–67)
Previous treatment	20 (12.7%)
Lesion size (mean ± SD) in disc area	3.11 ± 2.12
Lesion type (n [%])	
Predominantly classic	60 (38.2)
Minimally classic	37 (23.6)
Occult lesions	60 (38.2)
Disease status (mean [range])	
Central retinal/lesion thickness (μm)	456 (186–1316)
Foveal thickness (μm)	327 (116–1081)
Best-corrected visual acuity (ETDRS letters)	56 (27–83)

ETDRS = Early Treatment of Diabetic Retinopathy Study; F = Female; M = Male; SD = standard deviation.

Results

Patient Disposition

Of 159 patients who were randomized, 157 were treated and 134 (85.4%) completed 52 weeks in the study (Table 1 available online at http://aaojournal.org). For the 23 patients (14.6%) who were withdrawn before completion of 52 weeks, 6 (3.8%) withdrawals were at the request of the patient.

Baseline Characteristics

The study population was representative of the AMD population in the United States. Patients ranged in age from 53 to 94 years (mean, 78.3) and the majority were women (62%; Table 2). The mean time from diagnosis was 3.9 months (range, 0–67), and 12.7% of patients had received previous treatment. The distribution of CNV lesions was 38.2% predominantly classic, 23.6% minimally classic, and 38.2% occult with no classic. The treatment groups were well-balanced overall for baseline disease severity, but mean CR/LT and mean foveal thickness were somewhat higher in the 4 mg q12wk group (Table 3).

Change in Central Retinal/Lesion Thickness

The primary efficacy endpoint of the study was mean change in CR/LT at week 12. In all treatment groups combined, the significant decrease from baseline in CR/LT observed at week 12 (−119 μm) was maintained to week 52 (−130 μm; $P<0.0001$) after 40 weeks of PRN dosing with VEGF Trap-Eye (Fig 3A). The decrease in CR/LT was also maintained in all individual dosing groups after PRN dosing and was significant compared with baseline values. The greatest decreases in CR/LT at week 52 versus baseline occurred in the 4 mg q12wk group (−161 μm; $P = 0.0002$) and the 2 mg q4wk group (−143 μm; $P<0.0001$; Fig 3B).

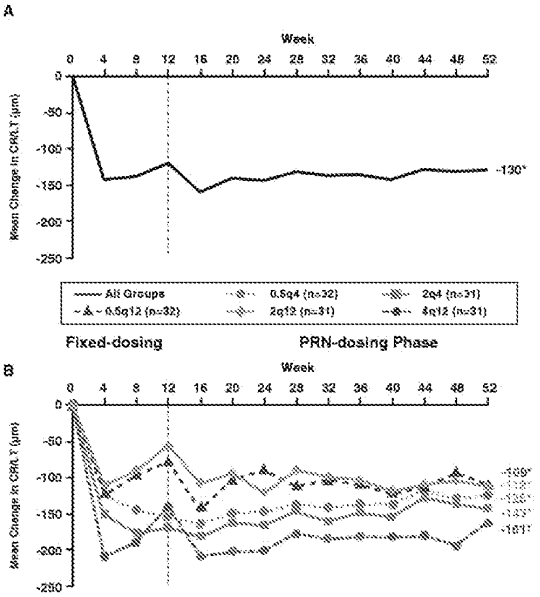


Figure 3. Mean change in central retinal/lesion (CR/LT) thickness in (**A**) all treatment groups combined and (**B**) individual treatment groups. The CR/LT was measured with optical coherence tomography. Change in CR/LT from baseline at 12 weeks was the primary study endpoint. In the combined treatment group, a significant (*$P<0.0001$) decrease in CR/LT at week 12 was maintained to week 52 (−130 μm). The decrease in CR/LT in individual treatment groups was maintained between weeks 12 and 52 with PRN dosing was significant (*$P<0.0001$; †$P = 0.0002$) compared with baseline values. The last-observation-carried-forward method was used to impute missing data. CR/LT = central retina/lesion thickness; PRN = as-needed; 0.5q4 = 0.5 mg every 4 weeks; 2q4 = 2 mg every 4 weeks; 0.5q12 = 0.5 mg every 12 weeks; 2q12 = 2 mg every 12 weeks; 4q12 = 4 mg every 12 weeks.

Change in Angiographic Measures

Fluorescein angiography (FA) was performed at baseline and at weeks 4, 12, 24, 36, and 48. In all groups combined and in each treatment group, there were no significant changes in total lesion

Table 3. Baseline Disease Status by Treatment Group

Mean (Range)	0.5q4 (n = 32)	2q4 (n = 31)	0.5q12 (n = 32)	2q12 (n = 31)	4q12 (n = 31)	All Groups (n = 157)
CR/LT (μm)	434 (282–710)	453 (232–960)	442 (186–762)	447 (265–948)	507 (240–1316)	456 (186–1316)
Foveal thickness (μm)	329 (212–509)	307 (171–524)	319 (116–559)	334 (186–762)	360 (177–1081)	327* (116–1081)
BCVA (ETDRS letters)	54 (27–76)	58 (32–83)	56 (30–72)	57 (32–72)	53 (28–80)	56 (27–83)

BCVA = best-corrected visual acuity; CR/LT = central retinal/lesion thickness; ETDRS = Early Treatment of Diabetic Retinopathy Study; q = every.
*In all groups (n = 157), 25 patients at week 52 showed foveal thickness measurements of <150 μm.

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size from baseline to week 12 and week 48 (data not shown). The decrease in total lesion size for the 2 mg q4wk group at week 12 (-0.75 mm^2; -0.30 disc area [DA]) and week 48 (-1.75 mm^2; -0.69 DA; $P<0.04$) achieved significance.

The area of CNV (defined as classic and/or occult CNV demonstrating angiographic evidence of late leakage or pooling of dye) was also measured. In all treatment groups combined, PRN treatment with VEGF Trap-Eye resulted in a consistent decrease in the CNV size versus baseline at weeks 12 and 48, with a decrease of -2.21 mm^2 (-0.87 DA) at week 48 ($P<0.001$; Fig 4A, available online at http://aaojournal.org). All treatment groups other than the 0.5 mg q12wk group experienced a decrease in active CNV size at 48 weeks (-1.42 to -3.41 mm^2; -0.56 to -1.34 DA), with the greatest reduction in the 2 mg q4wk group (-3.41 mm^2, -1.34 DA; $P<0.001$; Fig 4B).

Change in Visual Acuity

The significant improvement from baseline in BCVA that was noted at 12 weeks was maintained through the PRN dosing phase to week 52. The combining of all treatment groups showed a mean gain of 5.7 letters at week 12 and 5.3 letters at week 52 ($P<0.0001$ vs baseline; Fig 5A). The greatest improvement in BCVA occurred in the 2 mg q4wk treatment group, with a mean increase of 8.3 letters at week 12 and of 9.0 letters at week 52 ($P<0.0001$ vs baseline; Fig 5B). Visual acuity improvements compared with baseline were also maintained in all other treatment groups at week 52.

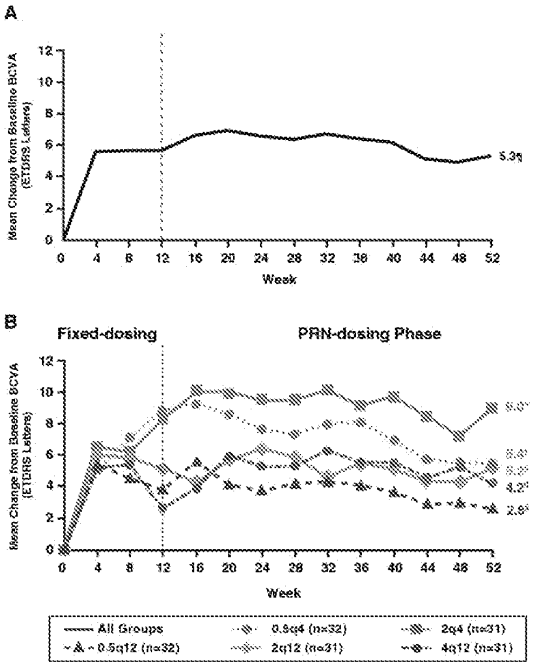


Figure 5. Mean change in best-corrected visual acuity (BCVA) in (A) all treatment groups combined and (B) individual treatment groups. The BCVA was assessed with the Early Treatment of Diabetic Retinopathy Study protocol at 4 m. Significant improvements from baseline in BCVA were noted in all treatment groups combined at week 12 (5.7 letters) and were maintained to week 52 (5.3 letters; [¶]$P<0.0001$). The 2 mg q4wk group showed the greatest gain in BCVA at 12 weeks, which was maintained to 52 weeks (9.0 letters; *$P<0.0001$; [†]$P = 0.085$; [‡]$P = 0.0412$; [§]$P = 0.0154$; and [‖]$P = 0.344$ for individual groups versus baseline). The last-observation-carried-forward method was used to impute missing data. ETDRS = Early Treatment of Diabetic Retinopathy Study; PRN = as-needed.

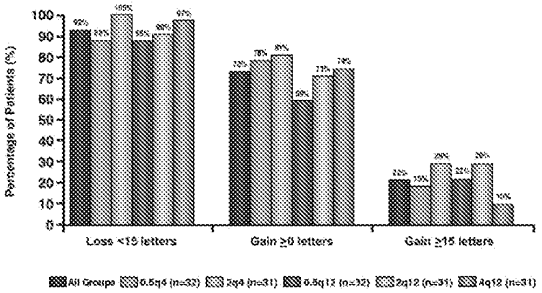


Figure 6. Visual acuity changes at week 52. The proportions of patients who avoided moderate vision loss (≥15 letters), had an improvement in visual acuity (gain of ≥0 letters), or had a significant vision gain (≥15 letters) in the treatment groups combined and individual dosing groups at week 52 are shown. In the treatment groups combined, only 8% of patients experienced moderate loss of vision, whereas 22% showed a significant gain in vision of ≥15 letters at week 52 after 40 weeks of as-needed (PRN) dosing. The last-observation-carried-forward method was used to impute missing data. 0.5q4 = 0.5 mg every 4 weeks; 2q4 = 2 mg every 4 weeks; 0.5q12 = 0.5 mg every 12 weeks; 2q12 = 2 mg every 12 weeks; 4q12 = 4 mg every 12 weeks.

Frequency of Patients with Visual Acuity Changes

In all treatment groups combined, moderate loss of vision (loss of ≥15 letters) was avoided in 98% of patients at week 12 and 92% at week 52 after treatment with VEGF Trap-Eye. In the individual treatment groups, 88% to 100% of patients avoided moderate loss of vision at week 52 (Fig 6). Overall, 12 patients experienced moderate vision loss at week 52, including 4 in the 0.5 mg q4wk group, 4 in the 0.5 mg q12wk group, 3 in the 2 mg q12wk group, and 1 in the 4 mg q12wk group. None of the patients in the 2 mg q4wk group experienced moderate vision loss.

Stabilization or improvement in visual acuity (gain of ≥0 letters) occurred in 74.5% of patients in all treatment groups combined at week 12 and in 73% of patients at week 52. In the individual treatment groups, the proportion of patients experiencing stable or improved visual acuity ranged between 59% and 81%, with the highest proportion in the 2 mg q4wk group. Overall, these proportions remained steady from week 12 to week 52.

The frequency of patients in all treatment groups combined with a significant gain in vision (≥15 letters) was 18.5% at week 12 and 22% at week 52. Groups treated with 2 mg, either monthly or quarterly, had the highest frequency of patients with significant visual gain (26% and 16%, respectively, at week 12, and 29% in each group at week 52).

The proportion of patients with ≤20/200 vision in all treatment groups combined was 11.5% at baseline and remained stable at 10.8% at week 52. The proportion of patients with ≥20/40 vision increased from baseline (15%) to week 52 (41%) for all groups combined, with the 0.5mg q4wk and 2mg q4wk groups increasing from 13% and 16% to 47% and 45%, respectively (Fig 7, available online at http://aaojournal.org).

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Table 4. Retreatment Outcomes with VEGF Trap-Eye

Treatment Regimen	Mean No. of Injections over PRN Phase (Weeks 12–52)	Mean No. of Days to First Injection over PRN Phase (Weeks 12–52)	Median No. of Days to First Injection over PRN Phase (Months 3–12)
0.5 mg q4	2.52	102	85
2 mg q4	1.55	160	150
0.5 mg q12	1.84	133	86
2 mg q12	2.48	113	86
4 mg q12	1.7	138	111
All groups	2.01	129	110

PRN = as-needed.

Reinjection Outcomes

Beginning at week 16, patients were evaluated monthly for the need for reinjection. Over the 40-week PRN dosing phase, the mean number of reinjections received for all groups combined was 2.01, ranging from 1.55 injections in the 2 mg q4wk group to 2.52 in the 0.5 mg q4wk group (Table 4). During the PRN dosing phase, 29 patients (19%) did not receive any injections of VEGF Trap-Eye, 45% received 1 to 2 injections, and only 5% of patients received ≥5 injections (Fig 8). The most common reason for retreatment was the presence of persistent fluid on OCT examination (63%); in 26% of patients who needed retreatment, a new or persistent leak was noted on FA, and 25% of patients had a loss of BCVA of ≥5 ETDRS letters with recurrent fluid on OCT (Table 5 available online at http://aaojournal.org).

The average time from the last mandatory injection at week 12 to the first PRN injection was 129 days for all treatment groups combined. The longest initial treatment-free interval was in the 2 mg q4wk group (160 days; Table 4). This calculation accounts for the 29 patients who were not reinjected by assigning them a reinjection time at 52 weeks and is therefore an underestimate of the true mean. Kaplan–Meier analysis of the time to first reinjection showed a median time to reinjection for all groups combined of 110 days. The longest median time to reinjection was 150 days in the 2 mg q4wk group (Fig 9, available online at http://aaojournal.org; Table 4).

Safety

The types and frequencies of ocular AEs occurring in the study eye were consistent with AEs previously reported with intravitreal anti-VEGF treatment and were generally related to the intravitreal injection procedure (Table 6). In all treatment groups combined, conjunctival hemorrhage (38.2%) was the most frequently reported AE in the study eye. Most ocular AEs in the study eye were mild (58%), with 4 events considered severe (conjunctival hemorrhage, reduced visual acuity, uveitis, and increased IOP). Seven patients (4.5%) had an increase in IOP in the study eye that was considered related to study treatment, but the increased IOP was not considered an SAE and did not lead to withdrawal of patients from the study. Most ocular events that occurred in the fellow eye were mild (41.4%) or moderate (12.7%); the most frequently reported AE in the fellow eye was vitreous detachment (8.9%).

The number of patients with systemic AEs was similar among treatment groups. The most commonly reported systemic AEs were urinary tract infection (10.2%), bronchitis (9.6%) and upper respiratory tract infection (9.6%). A total of 58 systemic SAEs were reported in 35 patients, but none of the events was deemed to be related to study treatment. Two deaths occurred during the study, one from pancreatic carcinoma and the other from preexisting pulmonary hypertension. Four ocular SAEs were reported: Culture-negative endophthalmitis/uveitis (0.5 mg q4wk group) and decreased visual acuity (0.5 mg q12wk group) in the study eye and increased IOP (4 mg q12wk group) and retinal detachment (0.5 mg q12wk group) in the fellow eye. Stroke was reported in 1 patient in the 0.5 mg q4wk group who had a history of stroke. No cases of vascular death were reported in this study.

Adverse events resulted in withdrawal of 7 patients from the

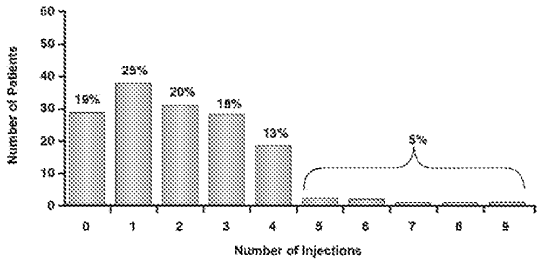


Figure 8. Number of injections after loading phase. Numbers of patients receiving 0 to 9 injections of VEGF Trap-Eye during the as-needed dosing period are shown. Overall, 19% of patients required no retreatment, 45% received 1 or 2 retreatments, and only 5% required 5 to 9 retreatments. VEGF = vascular endothelial growth factor.

Table 6. Ocular Adverse Events in the Study Eye (Frequency ≥5% in All Groups Combined*)

Adverse Event	Number (n)	Percent (%)
Conjunctival hemorrhage	60	38.2
Increased IOP (transient postinjection)	29	18.5
Refraction disorder	26	16.6
Retinal hemorrhage	22	14.0
Visual acuity reduced (patient reported)	21	13.4
Vitreous detachment	18	11.5
Eye pain	15	9.6
Vitreous floaters	14	8.9
Detachment of retinal pigment epithelium	12	7.6
Retinal edema	10	6.4
Visual disturbance	8	5.1
Blepharitis	8	5.1
Subretinal fibrosis	8	5.1

IOP = intraocular pressure.
*Patients receiving treatment with VEGF Trap-Eye (n = 157).

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study: Three ocular (retinal edema and retinal hemorrhage in the study eye and increased IOP in the fellow eye) and 4 systemic (non-Hodgkin's lymphoma, hip fracture, colon cancer, and bronchitis). There were no differences in frequency of AEs or SAEs between the treatment groups.

Discussion

Among patients with neovascular AMD, PRN dosing with VEGF Trap-Eye maintained efficacy established during a 12-week monthly or quarterly fixed-dosing phase for an additional 40 weeks. For all groups combined, a clinically significant improvement in visual acuity achieved at 12 weeks (5.7-letter gain) was maintained to 52 weeks (5.3-letter gain), accompanied by a decrease in CR/LT (−119 μm at week 12 and −130 μm at week 52). Among patients in all treatment groups combined, 22% experienced a gain of ≥15 letters and only 8% of patients had a loss of ≥15 letters at the end of the study period. These improvements were achieved with an average of only 2 additional injections of VEGF Trap-Eye over the 40-week PRN phase; notably, 44% of patients required either no retreatment or only 1 reinjection, suggesting a long duration of effect in these patients. Repeated intravitreal dosing of VEGF Trap-Eye was generally safe and well tolerated. The overall safety profile was similar to that previously reported with other intravitreal anti-VEGF agents.

Anti-VEGF treatment offers the hope of improved vision and is now the standard of care for neovascular AMD; however, the need for frequent monitoring and treatment places a significant burden on both the patient and the clinician. In addition, the injection procedure itself has associated risks, which, although rare, may be increased with repeated injections. Hence, a less frequent dosing regimen that is maximally effective in preserving or improving visual acuity is a desirable goal of anti-VEGF treatment for AMD.

With VEGF Trap-Eye, the improvements in visual acuity achieved with a 12-week fixed dosing schedule were maintained to week 52 with an average of 2 injections over the 9-month period after fixed dosing. In the 2 mg q4wk group, ≥50% of patients remained injection free for 150 days after week 12. The best visual acuity outcomes to date with ranibizumab have been achieved with 3 consecutive monthly injections, followed by continuous monthly injections.[13,15,32] Less frequent dosing with ranibizumab using both fixed and PRN schedules has been investigated. In the 2-year Phase 3b, Multicenter, Randomized, Double-masked, Sham Injection-controlled Study of the Safety and Efficacy of Ranibizumab (PIER), study a regimen of 3 consecutive monthly injections followed by fixed quarterly injections provided vision gains at 3 months that were comparable with those in the pivotal clinical studies; however, these gains declined with quarterly dosing and returned to baseline levels at 12 months.[21] Results of a phase 3b study designed to evaluate the long-term effect of ranibizumab in patients with all subtypes of neovascular AMD (The Safety Assessment of Intravitreal Lucentis for AMD [SAILOR]) suggested that treating patients with ranibizumab on a PRN basis was less effective than monthly dosing, although retreatment criteria and follow-up schedules in this study were less well-defined and investigator determined.[33] In the open-label HORIZON extension study, patients received PRN dosing with ranibizumab after 2 years of monthly dosing, resulting in a decline in visual acuity gain at years 3 and 4 (Invest Ophthalmol Vis Sci 50 [Suppl]:3093, 2009).

The 52-week results of the CLEAR-IT 2 study suggest that the efficacy of VEGF Trap-Eye, established with an initial fixed-dosing regimen, is maintained with PRN dosing and that VEGF Trap-Eye has an extended duration of action. The average time from last mandatory injection to first PRN injection for all groups combined was >4 months and for the 2 mg q4wk group, >5 months. The high binding affinity of VEGF Trap-Eye, its presumed long intravitreal half-life, and activity against multiple VEGF family members, including PlGF, may contribute to more sustained and comprehensive VEGF suppression than may be achieved with currently used anti-VEGF agents. Based on its binding affinity and estimated intravitreal half-life in humans, a mathematical model has predicted that, after intravitreal injection, VEGF Trap-Eye would maintain biological activity for 10 to 12 weeks, whereas ranibizumab would maintain such activity for 30 days, supporting the concept of less frequent dosing with VEGF Trap-Eye.[30]

By the end of the fixed-dosing phase at week 12, VEGF Trap-Eye showed a significant improvement in visual acuity from baseline, and this efficacy was maintained to week 52. However, patients randomized to monthly injections during the fixed dosing phase had a trend toward improved visual acuity outcomes at 52 weeks compared with those randomized to quarterly injections. This difference was apparent at 12 weeks and was maintained throughout the 40-week PRN phase, despite the fact that all patients were treated as often as necessary during this phase. Therefore, initial sequential monthly loading doses seem to provide better control of neovascular leakage and lead to superior gains in visual acuity that can be subsequently maintained with less frequent dosing.

Ongoing phase 3 studies (VEGF Trap-Eye: Investigation of Efficacy and Safety in wet Age-Related Macular Degeneration, VIEW-1 and VIEW-2) are evaluating VEGF Trap-Eye doses of 0.5 and 2 mg every 4 weeks and 2 mg every 8 weeks after 3 initial monthly doses, compared with ranibizumab 0.5 mg every 4 weeks. The rationale for the 2 mg q8wk dose was based on an observation from the fixed-dosing phase of CLEAR-IT 2: At 8 weeks, the improvement in BCVA after a single 2-mg dose was similar to that obtained with 2 mg dosed monthly to 8 weeks and that the effect in visual acuity gain for the single dose groups began to wane by week 12.

After the first year of treatment on the above schedule, patients in the VIEW-1 and -2 studies will be evaluated monthly and treated on a PRN basis. To prevent undertreatment, the PRN dosing schedule during the second year of the study does not allow any patient to remain untreated for >12 weeks. Although many patients in the CLEAR-IT 2 study did maintain initial visual acuity gains for >12 weeks, the design of the phase 3 program is meant to optimize visual improvements for all patients by using this capped

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PRN dosing schedule. These studies should provide further information on the duration and extent of the clinical benefit of VEGF Trap-Eye.

In conclusion, PRN dosing of VEGF Trap-Eye after 12 weeks of monthly or quarterly fixed dosing maintained clinically and statistically significant improvements in vision and retinal thickness to week 52 in patients with neovascular AMD, with a low frequency of reinjection. VEGF Trap-Eye was generally well-tolerated, with a safety profile similar to that reported with other intravitreally administered anti-VEGF agents.

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Footnotes and Financial Disclosures

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initial work were those that were underrepresented or excluded. Indeed, eyes at the extreme of axial length and keratometry, high astigmatism, postrefractive patients, and eyes with previous surgery (e.g., penetrating keratoplasty) are notorious for difficulty in estimation of the postoperative IOL position. Analysis of these groups may demonstrate significant improvement to a very difficult problem.

Another question, and the most difficult to address, is how to address the postoperative standard of care for our patients. We believe that there is enough evidence at this time to suggest evaluating the postoperative refraction of the first eye in most patients. Refractive stability after cataract surgery occurs within a few weeks, and thus, waiting for the second eye should be considered. Our current practice is to wait at least 3 to 4 weeks between surgeries, which allows for analysis of the refractive result among other postoperative factors. Bilateral simultaneous surgery, which has been advocated by some investigators, would obviously preclude a patient from these potential advantages. Finally, we thank the authors of these 2 large retrospective studies, which will further enhance our collective outcomes as we strive for postoperative refractive perfection.

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Erratum

With apologies from the authors, the legend for Figure 5 in "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;1098-106) contained errors in the order of some of the *P* values. Below is the corrected legend with changes in boldface.

Figure 5. Mean change in best-corrected visual acuity (BCVA) in (**A**) all treatment groups combined and (**B**) individual treatment groups. The BCVA was assessed with the Early Treatment of Diabetic Retinopathy Study protocol at 4 m. Significant improvements from baseline in BCVA were noted in all treatment groups combined at week 12 (5.7 letters) and were maintained to week 52 (5.3 letters; $^{\P}P < 0.0001$). The 2 mg q4wk group showed the greatest gain in BCVA at 12 weeks, which was maintained to 52 weeks (9.0 letters; $^{*}P < 0.0001$; $^{\dagger}P = 0.085$; $^{\ddagger}\mathbf{P = 0.344}$; $^{\S}\mathbf{P = 0.0412}$; **and** $^{\|}\mathbf{P = 0.0154}$ for individual groups versus baseline). The last-observation-carried-forward method was used to impute missing data. ETDRS = Early Treatment of Diabetic Retinopathy Study; PRN = as-needed.

Mylan Exhibit 1017
Mylan v. Regeneron, IPR2022-01226
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Samsung Bioepis Exhibit 1017, pg. 991

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History of Changes for Study: NCT00320775

Safety and Tolerability Study of Intravitreal VEGF-Trap Administration in Patients With Neovascular AMD

Latest version (submitted March 16, 2015) on ClinicalTrials.gov

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3	○	○	July 25, 2007	Recruitment Status, Study Status and Contacts/Locations

Version	A	B	Submitted Date	Changes
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7	○	○	January 25, 2011	Study Status
8	◉	◉	June 8, 2011	Study Status
9	○	○	March 16, 2015	Sponsor/Collaborators, Study Status and References

Compare

Comparison Format: ◉ Merged ○ Side-by-Side

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Study NCT00320775

Submitted Date: June 8, 2011 (v8)

Study Identification

Unique Protocol ID: VGFT-OD-0502

Brief Title: Safety and Tolerability Study of Intravitreal VEGF-Trap Administration in Patients With Neovascular AMD

Official Title: An Exploratory Study of the Safety, Tolerability and Biological Effect of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification: June 2011

Overall Status:	Completed
Study Start:	June 2005
Primary Completion:	June 2008 [Actual]
Study Completion:	August 2008 [Actual]
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Last Update Submitted that Met QC Criteria:	June 8, 2011
Last Update Posted:	June 10, 2011 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	Bayer

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	No

Study Description

Brief Summary:	The purpose of this trial is to assess the ocular and systemic safety and tolerability of a single intravitreal injection of VEGF Trap in patients with subfoveal choroidal neovascularization (CNV) due to AMD.
Detailed Description:	This study consists of three parts, Part A, Part B and Part C. Part A is a dose escalation. Part B was terminated early. The (one) subject who received Macugen is not discussed in this website. Part C had

subjects receive one of two doses of VEGF Trap (0.15 mg or 4.0 mg).

This is the first study in which human subjects received intravitreal injections of VEGF Trap in a study eye.

Conditions

Conditions:	Macular Degeneration
Keywords:	Neovascular Age-Related Macular Degeneration

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 1
Interventional Study Model:	Parallel Assignment
Number of Arms:	3
Masking:	None (Open Label)
Allocation:	Randomized
Enrollment:	51 [Actual]

Arms and Interventions

Arms	Assigned Interventions

Arms	Assigned Interventions
Experimental: Part A Part A: An open label study in which six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. The total volume of each injection will be 100 µL. Enrollment in new dose levels will not begin until all patients in the preceding dose level have completed Visit 5 (Day 15).	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

Arms	Assigned Interventions
Active Comparator: Part B Part B: A controlled, prospective, randomized, double-masked study in which up to 30 subjects meeting eligibility criteria will be randomly assigned in a 1:1 ratio to receive a single ITV injection of2.0 mg/eye VEGF Trap (or the MTD if reached prior to 2.0 mg) followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Enrollment into Part B will begin 2 weeks after the last subject to receive the 2.0 mg/eye dose in Part A has been observed for 15 days and it has been determined that the safety profile of VEGF Trap at this dose level is adequate to support expansion of dosing at this dose level. The dose of pegaptanib sodium will be 0.3 mg, according to the package insert.	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

Arms	Assigned Interventions
Active Comparator: Part C Part C: A controlled, prospective, randomized, double-masked study in which approximately 30 subjects meeting eligibility criteria will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. Initiation of Part C is contingent upon the 4.0 mg dose being adequately tolerated in Part A.	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

Outcome Measures

Primary Outcome Measures:

1. Safety and tolerability, bioeffect
   From baseline to Day 43

Secondary Outcome Measures:

2. The effect of VEGF Trap administration on excess central retinal/lesion thickness
   From baseline to Day 43
3. Best-corrected Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuity
   From baseline to Day 43
4. Extent of CNV leakage

From baseline to Day 43

5. Anti-VEGF Trap antibodies in the systemic circulation
   From baseline to Day 43
6. Plasma levels of VEGF Trap
   From baseliene to Day 43

Eligibility

Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Subfoveal CNV secondary to AMD.
- Central retinal/lesion thickness ≥ 250µm as measured by optical coherence tomography (OCT).
- ETDRS best-corrected visual acuity of:
  - 20/40 (73 letters) or worse
- Clear ocular media and clear lens(es) to permit good quality stereoscopic fundus photography.

Exclusion Criteria:

- Prior treatment with VEGF Trap, bevacizumab or ranibizumab.
- Any investigational agent within 12 weeks of Visit 2 (Day 1).
- Presence of other causes of CNV.
- Active ocular infection.

Contacts/Locations

Study Officials: Avner Ingerman, MD
Study Director
Regeneron Pharmaceuticals

Locations: **United States, Arizona**

Retina Centers, PC
Tuscon, Arizona, United States, 85704

United States, California

Loma Linda University Health Care
Loma Linda, California, United States, 92354

United States, Illinois

University of Chicago
Chicago, Illinois, United States, 60637

United States, Maryland

Johns Hopkins Hospital School of Medicine
Baltimore, Maryland, United States, 21287

United States, North Carolina

Charlotte Eye, Ear, Nose & Throat Asssociates
Charlotte, North Carolina, United States, 28120

United States, Oklahoma

Dean A. McGee Eye Institute
Oklahoma City, Oklahoma, United States, 73104

United States, Pennsylvania

Retina Diagnostic and Treatment Assoc., LLC
Philadelphia, Pennsylvania, United States, 19107

United States, Tennessee

Retina-Vitreous Associates, P.C.
Nashville, Tennessee, United States, 37203

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ClinicalTrials.gov archive

History of Changes for Study: NCT00320775

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9	○	○	March 16, 2015	Sponsor/Collaborators, Study Status and References

Compare

Comparison Format: ◉ Merged ○ Side-by-Side

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Study NCT00320775

Submitted Date: January 25, 2011 (v7)

Study Identification

Unique Protocol ID: VGFT-OD-0502

Brief Title: Safety and Tolerability Study of Intravitreal VEGF-Trap Administration in Patients With Neovascular AMD

Official Title: An Exploratory Study of the Safety, Tolerability and Biological Effect of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification: January 2011

Overall Status:	Completed
Study Start:	June 2005
Primary Completion:	June 2008 [Actual]
Study Completion:	August 2008 [Actual]
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Last Update Submitted that Met QC Criteria:	January 25, 2011
Last Update Posted:	January 26, 2011 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	Bayer

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	No

Study Description

Brief Summary:	The purpose of this trial is to assess the ocular and systemic safety and tolerability of a single intravitreal injection of VEGF Trap in patients with subfoveal choroidal neovascularization (CNV) due to AMD.
Detailed Description:	This study consists of three parts, Part A, Part B and Part C. Part A is a dose escalation. Part B was terminated early. The (one) subject who received Macugen is not discussed in this website. Part C had

subjects receive one of two doses of VEGF Trap (0.15 mg or 4.0 mg).

This is the first study in which human subjects received intravitreal injections of VEGF Trap in a study eye.

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Conditions:	Macular Degeneration
Keywords:	Neovascular Age-Related Macular Degeneration

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 1
Interventional Study Model:	Parallel Assignment
Number of Arms:	3
Masking:	None (Open Label)
Allocation:	Randomized
Enrollment:	51 [Actual]

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Experimental: Part A Part A: An open label study in which six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. The total volume of each injection will be 100 µL. Enrollment in new dose levels will not begin until all patients in the preceding dose level have completed Visit 5 (Day 15).	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

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From baseline to Day 43

5. Anti-VEGF Trap antibodies in the systemic circulation
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Eligibility

Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

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Contacts/Locations

Study Officials: Avner Ingerman, MD
Study Director
Regeneron Pharmaceuticals

Locations: **United States, Arizona**

Retina Centers, PC
Tuscon, Arizona, United States, 85704

United States, California

Loma Linda University Health Care
Loma Linda, California, United States, 92354

United States, Illinois

University of Chicago
Chicago, Illinois, United States, 60637

United States, Maryland

Johns Hopkins Hospital School of Medicine
Baltimore, Maryland, United States, 21287

United States, North Carolina

Charlotte Eye, Ear, Nose & Throat Asssociates
Charlotte, North Carolina, United States, 28120

United States, Oklahoma

Dean A. McGee Eye Institute
Oklahoma City, Oklahoma, United States, 73104

United States, Pennsylvania

Retina Diagnostic and Treatment Assoc., LLC
Philadelphia, Pennsylvania, United States, 19107

United States, Tennessee

Retina-Vitreous Associates, P.C.
Nashville, Tennessee, United States, 37203

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U.S. National Library of Medicine

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History of Changes for Study: NCT00320775

Safety and Tolerability Study of Intravitreal VEGF-Trap Administration in Patients With Neovascular AMD

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9	○	○	March 16, 2015	Sponsor/Collaborators, Study Status and References

Compare

Comparison Format: ◉ Merged ○ Side-by-Side

Scroll up to access the controls

Study NCT00320775

Submitted Date: January 26, 2010 (v6)

Study Identification

Unique Protocol ID: VGFT-OD-0502

Brief Title: Safety and Tolerability Study of Intravitreal VEGF-Trap Administration in Patients With Neovascular AMD

Official Title: An Exploratory Study of the Safety, Tolerability and Biological Effect of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification: January 2010

Overall Status:	Completed
Study Start:	June 2005
Primary Completion:	June 2008 [Actual]
Study Completion:	August 2008 [Actual]
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Last Update Submitted that Met QC Criteria:	January 26, 2010
Last Update Posted:	January 27, 2010 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	Bayer

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	No

Study Description

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Keywords:	Neovascular Age-Related Macular Degeneration

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Primary Purpose:	Treatment
Study Phase:	Phase 1
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Experimental: Part A Part A: An open label study in which six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. The total volume of each injection will be 100 µL. Enrollment in new dose levels will not begin until all patients in the preceding dose level have completed Visit 5 (Day 15).	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

Arms	Assigned Interventions
Active Comparator: Part B Part B: A controlled, prospective, randomized, double-masked study in which up to 30 subjects meeting eligibility criteria will be randomly assigned in a 1:1 ratio to receive a single ITV injection of2.0 mg/eye VEGF Trap (or the MTD if reached prior to 2.0 mg) followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Enrollment into Part B will begin 2 weeks after the last subject to receive the 2.0 mg/eye dose in Part A has been observed for 15 days and it has been determined that the safety profile of VEGF Trap at this dose level is adequate to support expansion of dosing at this dose level. The dose of pegaptanib sodium will be 0.3 mg, according to the package insert.	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

Arms	Assigned Interventions
Active Comparator: Part C Part C: A controlled, prospective, randomized, double-masked study in which approximately 30 subjects meeting eligibility criteria will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. Initiation of Part C is contingent upon the 4.0 mg dose being adequately tolerated in Part A.	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

Outcome Measures

Primary Outcome Measures:

1. Safety and tolerability, bioeffect
   From baseline to Day 43

Secondary Outcome Measures:

2. The effect of VEGF Trap administration on excess central retinal/lesion thickness
   From baseline to Day 43
3. Best-corrected Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuity
   From baseline to Day 43
4. Extent of CNV leakage

From baseline to Day 43

5. Anti-VEGF Trap antibodies in the systemic circulation
   From baseline to Day 43
6. Plasma levels of VEGF Trap
   From baseliene to Day 43

Eligibility

Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Subfoveal CNV secondary to AMD.
- Central retinal/lesion thickness ≥ 250µm as measured by optical coherence tomography (OCT).
- ETDRS best-corrected visual acuity of:
  - 20/40 (73 letters) or worse
- Clear ocular media and clear lens(es) to permit good quality stereoscopic fundus photography.

Exclusion Criteria:

- Prior treatment with VEGF Trap, bevacizumab or ranibizumab.
- Any investigational agent within 12 weeks of Visit 2 (Day 1).
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Contacts/Locations

Study Officials: Avner Ingerman, MD
Study Director
Regeneron Pharmaceuticals

Locations: **United States, Arizona**

Retina Centers, PC
Tuscon, Arizona, United States, 85704

United States, California

Loma Linda University Health Care
Loma Linda, California, United States, 92354

United States, Illinois

University of Chicago
Chicago, Illinois, United States, 60637

United States, Maryland

Johns Hopkins Hospital School of Medicine
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United States, North Carolina

Charlotte Eye, Ear, Nose & Throat Asssociates
Charlotte, North Carolina, United States, 28120

United States, Oklahoma

Dean A. McGee Eye Institute
Oklahoma City, Oklahoma, United States, 73104

United States, Pennsylvania

Retina Diagnostic and Treatment Assoc., LLC
Philadelphia, Pennsylvania, United States, 19107

United States, Tennessee

Retina-Vitreous Associates, P.C.
Nashville, Tennessee, United States, 37203

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History of Changes for Study: NCT00320775

Safety and Tolerability Study of Intravitreal VEGF-Trap Administration in Patients With Neovascular AMD

Latest version (submitted March 16, 2015) on ClinicalTrials.gov

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3	○	○	July 25, 2007	Recruitment Status, Study Status and Contacts/Locations

Version	A	B	Submitted Date	Changes
4	○	○	January 23, 2009	Recruitment Status, Study Status, Study Design, Arms and Interventions, Contacts/Locations, Outcome Measures, Oversight, Sponsor/Collaborators and Study Identification
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6	○	○	January 26, 2010	Study Status
7	○	○	January 25, 2011	Study Status
8	○	○	June 8, 2011	Study Status
9	○	○	March 16, 2015	Sponsor/Collaborators, Study Status and References

Compare

Comparison Format: ◉ Merged ○ Side-by-Side

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Study NCT00320775

Submitted Date: April 29, 2009 (v5)

Study Identification

Unique Protocol ID: VGFT-OD-0502

Brief Title: Safety and Tolerability Study of Intravitreal VEGF-Trap Administration in Patients With Neovascular AMD

Official Title: An Exploratory Study of the Safety, Tolerability and Biological Effect of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification: April 2009

Overall Status:	Completed
Study Start:	June 2005
Primary Completion:	June 2008 [Actual]
Study Completion:	August 2008 [Actual]
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Last Update Submitted that Met QC Criteria:	April 29, 2009
Last Update Posted:	April 30, 2009 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	Bayer

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	No

Study Description

Brief Summary:	The purpose of this trial is to assess the ocular and systemic safety and tolerability of a single intravitreal injection of VEGF Trap in patients with subfoveal choroidal neovascularization (CNV) due to AMD.
Detailed Description:	This study consists of three parts, Part A, Part B and Part C. Part A is a dose escalation. Part B was terminated early. The (one) subject who received Macugen is not discussed in this website. Part C had

subjects receive one of two doses of VEGF Trap (0.15 mg or 4.0 mg).

This is the first study in which human subjects received intravitreal injections of VEGF Trap in a study eye.

Conditions

Conditions:	Macular Degeneration
Keywords:	Neovascular Age-Related Macular Degeneration

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 1
Interventional Study Model:	Parallel Assignment
Number of Arms:	3
Masking:	None (Open Label)
Allocation:	Randomized
Enrollment:	51 [Actual]

Arms and Interventions

Arms	Assigned Interventions

Arms	Assigned Interventions
Experimental: Part A Part A: An open label study in which six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. The total volume of each injection will be 100 µL. Enrollment in new dose levels will not begin until all patients in the preceding dose level have completed Visit 5 (Day 15).	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

Arms	Assigned Interventions
Active Comparator: Part B Part B: A controlled, prospective, randomized, double-masked study in which up to 30 subjects meeting eligibility criteria will be randomly assigned in a 1:1 ratio to receive a single ITV injection of2.0 mg/eye VEGF Trap (or the MTD if reached prior to 2.0 mg) followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Enrollment into Part B will begin 2 weeks after the last subject to receive the 2.0 mg/eye dose in Part A has been observed for 15 days and it has been determined that the safety profile of VEGF Trap at this dose level is adequate to support expansion of dosing at this dose level. The dose of pegaptanib sodium will be 0.3 mg, according to the package insert.	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

Arms	Assigned Interventions
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Outcome Measures

Primary Outcome Measures:

1. Safety and tolerability, bioeffect
   From baseline to Day 43

Secondary Outcome Measures:

2. The effect of VEGF Trap administration on excess central retinal/lesion thickness
   From baseline to Day 43
3. Best-corrected Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuity
   From baseline to Day 43
4. Extent of CNV leakage

From baseline to Day 43

5. Anti-VEGF Trap antibodies in the systemic circulation
   From baseline to Day 43
6. Plasma levels of VEGF Trap
   From baseliene to Day 43

Eligibility

Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Subfoveal CNV secondary to AMD.
- Central retinal/lesion thickness ≥ 250µm as measured by optical coherence tomography (OCT).
- ETDRS best-corrected visual acuity of:
  - 20/40 (73 letters) or worse
- Clear ocular media and clear lens(es) to permit good quality stereoscopic fundus photography.

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- Prior treatment with VEGF Trap, bevacizumab or ranibizumab.
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Contacts/Locations

Study Officials: Avner Ingerman, MD
Study Director
Regeneron Pharmaceuticals

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Nashville, Tennessee, United States, 37203

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ClinicalTrials.gov archive

History of Changes for Study: NCT00320775

Safety and Tolerability Study of Intravitreal VEGF-Trap Administration in Patients With Neovascular AMD

Latest version (submitted March 16, 2015) on ClinicalTrials.gov

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3	○	○	July 25, 2007	Recruitment Status, Study Status and Contacts/Locations

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7	○	○	January 25, 2011	Study Status
8	○	○	June 8, 2011	Study Status
9	○	○	March 16, 2015	Sponsor/Collaborators, Study Status and References

Compare

Comparison Format: ◉ Merged ○ Side-by-Side

Scroll up to access the controls

Study NCT00320775

Submitted Date: January 23, 2009 (v4)

Study Identification

Unique Protocol ID: VGFT-OD-0502

Brief Title: Safety and Tolerability Study of Intravitreal VEGF-Trap Administration in Patients With Neovascular AMD

Official Title: An Exploratory Study of the Safety, Tolerability and Biological Effect of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification: January 2009

Overall Status:	Completed
Study Start:	June 2005
Primary Completion:	June 2008 [Actual]
Study Completion:	August 2008 [Actual]
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Last Update Submitted that Met QC Criteria:	January 23, 2009
Last Update Posted:	January 27, 2009 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	No

Study Description

Brief Summary:	The purpose of this trial is to assess the ocular and systemic safety and tolerability of a single intravitreal injection of VEGF Trap in patients with subfoveal choroidal neovascularization (CNV) due to AMD.
Detailed Description:	This study consists of three parts, Part A, Part B and Part C.

Conditions

Conditions: Macular Degeneration

Keywords: Neovascular Age-Related Macular Degeneration

Study Design

Study Type: Interventional

Primary Purpose: Treatment

Study Phase: Phase 1

Interventional Study Model: Parallel Assignment

Number of Arms: 3

Masking: None (Open Label)

Allocation: Randomized

Enrollment: 51 [Actual]

Arms and Interventions

Arms	Assigned Interventions

Arms	Assigned Interventions
Experimental: Part A Part A: An open label study in which six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. The total volume of each injection will be 100 µL. Enrollment in new dose levels will not begin until all patients in the preceding dose level have completed Visit 5 (Day 15).	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

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From baseline to Day 43

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Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

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Charlotte Eye, Ear, Nose & Throat Asssociates
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History of Changes for Study: NCT00320775

Safety and Tolerability of Intravitreal Administration of Vascular Endothelial Growth Factor (VEGF) Trap in Patients With Neovascular Age-Related Macular Degeneration (AMD)

Latest version (submitted March 16, 2015) on ClinicalTrials.gov

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9	○	○	March 16, 2015	Sponsor/Collaborators, Study Status and References

Compare

Comparison Format: ◉ Merged ○ Side-by-Side

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Study NCT00320775

Submitted Date: July 25, 2007 (v3)

Study Identification

Unique Protocol ID: VGFT-OD-0502

Brief Title: Safety and Tolerability of Intravitreal Administration of Vascular Endothelial Growth Factor (VEGF) Trap in Patients With Neovascular Age-Related Macular Degeneration (AMD)

Official Title: An Exploratory Study of the Safety, Tolerability and Biological Effect of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification:	July 2007
Overall Status:	Active, not recruiting
Study Start:	June 2005
Primary Completion:	
Study Completion:	
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Last Update Submitted that Met QC Criteria:	July 25, 2007
Last Update Posted:	July 27, 2007 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	

Study Description

Brief Summary:	The purpose of this trial is to assess the ocular and systemic safety and tolerability of a single intravitreal injection of VEGF Trap in patients with subfoveal choroidal neovascularization (CNV) due to AMD.

Detailed Description: This study consists of three parts, Part A, Part B and Part C.

Conditions

Conditions: Macular Degeneration

Keywords: Neovascular Age-Related Macular Degeneration

Study Design

Study Type: Interventional

Primary Purpose: Treatment

Study Phase: Phase 1

Interventional Study Model:

Number of Arms:

Masking: (masked roles unspecified)

Allocation: N/A

Enrollment: 96

Arms and Interventions

Intervention Details:

Drug: VEGF Trap

Outcome Measures

Primary Outcome Measures:

1. Safety and tolerability, bioeffect

Secondary Outcome Measures:

2. The effect of VEGF Trap administration on excess central retinal/lesion thickness
3. Best-corrected Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuity
4. Extent of CNV leakage
5. Anti-VEGF Trap antibodies in the systemic circulation
6. Plasma levels of VEGF Trap

Eligibility

Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Subfoveal CNV secondary to AMD.
- Central retinal/lesion thickness ≥ 250µm as measured by optical coherence tomography (OCT).
- ETDRS best-corrected visual acuity of:
  - 20/40 (73 letters) or worse
- Clear ocular media and clear lens(es) to permit good quality stereoscopic fundus photography.

Exclusion Criteria:

- Prior treatment with VEGF Trap, bevacizumab or ranibizumab.
- Any investigational agent within 12 weeks of Visit 2 (Day 1).
- Presence of other causes of CNV.
- Active ocular infection.

Contacts/Locations

Study Officials: Avner Ingerman, MD
Study Director

Locations: **United States, Arizona**

Tuscon, Arizona, United States, 85704

United States, California

Loma Linda, California, United States, 92354

United States, Illinois

Chicago, Illinois, United States, 60637

United States, Maryland

Baltimore, Maryland, United States, 21287

United States, North Carolina

Charlotte, North Carolina, United States, 28120

United States, Ohio

Cleveland, Ohio, United States, 44195

United States, Oklahoma

Oklahoma City, Oklahoma, United States, 73104

United States, Pennsylvania

Philadelphia, Pennsylvania, United States, 19107

United States, Tennessee

Nashville, Tennessee, United States, 37203

United States, Wisconsin

Madison, Wisconsin, United States, 53705

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History of Changes for Study: NCT00320775

Safety and Tolerability of Intravitreal Administration of Vascular Endothelial Growth Factor (VEGF) Trap in Patients With Neovascular Age-Related Macular Degeneration (AMD)

Latest version (submitted March 16, 2015) on ClinicalTrials.gov

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- Study edits or deletions are displayed in ~~red~~.
- Study additions are displayed in *green*.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	○	○	April 28, 2006	None (earliest Version on record)
2	◉	◉	October 3, 2006	Outcome Measures, Conditions, Study Status, Eligibility, Study Description and Study Identification

Version	A	B	Submitted Date	Changes
3	○	○	July 25, 2007	Recruitment Status, Study Status and Contacts/Locations
4	○	○	January 23, 2009	Recruitment Status, Study Status, Study Design, Arms and Interventions, Contacts/Locations, Outcome Measures, Oversight, Sponsor/Collaborators and Study Identification
5	○	○	April 29, 2009	Study Status, Arms and Interventions, Study Description and Sponsor/Collaborators
6	○	○	January 26, 2010	Study Status
7	○	○	January 25, 2011	Study Status
8	○	○	June 8, 2011	Study Status
9	○	○	March 16, 2015	Sponsor/Collaborators, Study Status and References

Compare

Comparison Format: ◉ Merged ○ Side-by-Side

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Study NCT00320775

Submitted Date: October 3, 2006 (v2)

Study Identification

Unique Protocol ID: VGFT-OD-0502

Brief Title: Safety and Tolerability of Intravitreal Administration of Vascular Endothelial Growth Factor (VEGF) Trap in Patients With Neovascular Age-Related Macular Degeneration (AMD)

Official Title: An Exploratory Study of the Safety, Tolerability and Biological Effect of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification:	April 2006
Overall Status:	Recruiting
Study Start:	June 2005
Primary Completion:	
Study Completion:	
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Last Update Submitted that Met QC Criteria:	October 3, 2006
Last Update Posted:	October 4, 2006 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	

Study Description

Brief Summary:	The purpose of this trial is to assess the ocular and systemic safety and tolerability of a single intravitreal injection of VEGF Trap in patients with subfoveal choroidal neovascularization (CNV) due to AMD.

Detailed Description: This study consists of three parts, Part A, Part B and Part C.

Conditions

Conditions: Macular Degeneration

Keywords: Neovascular Age-Related Macular Degeneration

Study Design

Study Type: Interventional

Primary Purpose: Treatment

Study Phase: Phase 1

Interventional Study Model:

Number of Arms:

Masking: (masked roles unspecified)

Allocation: N/A

Enrollment: 96

Arms and Interventions

Intervention Details:

Drug: VEGF Trap

Outcome Measures

Primary Outcome Measures:

1. Safety and tolerability, bioeffect

Secondary Outcome Measures:

2. The effect of VEGF Trap administration on excess central retinal/lesion thickness
3. Best-corrected Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuity
4. Extent of CNV leakage
5. Anti-VEGF Trap antibodies in the systemic circulation
6. Plasma levels of VEGF Trap

Eligibility

Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Subfoveal CNV secondary to AMD.
- Central retinal/lesion thickness ≥ 250µm as measured by optical coherence tomography (OCT).
- ETDRS best-corrected visual acuity of:
  - 20/40 (73 letters) or worse
- Clear ocular media and clear lens(es) to permit good quality stereoscopic fundus photography.

Exclusion Criteria:

- Prior treatment with VEGF Trap, bevacizumab or ranibizumab.
- Any investigational agent within 12 weeks of Visit 2 (Day 1).
- Presence of other causes of CNV.
- Active ocular infection.

Contacts/Locations

Central Contact: Regeneron
Email: VEGF.Trap@regeneron.com

Locations: **United States, Arizona**

[Recruiting]
Tuscon, Arizona, United States, 85704

United States, California

[Recruiting]
Loma Linda, California, United States, 92354

United States, Illinois

[Recruiting]

Chicago, Illinois, United States, 60637

United States, Maryland

[Recruiting]

Baltimore, Maryland, United States, 21287

United States, North Carolina

[Recruiting]

Charlotte, North Carolina, United States, 28120

United States, Ohio

[Recruiting]

Cleveland, Ohio, United States, 44195

United States, Oklahoma

[Recruiting]

Oklahoma City, Oklahoma, United States, 73104

United States, Pennsylvania

[Recruiting]

Philadelphia, Pennsylvania, United States, 19107

United States, Tennessee

[Recruiting]

Nashville, Tennessee, United States, 37203

United States, Wisconsin

[Recruiting]

Madison, Wisconsin, United States, 53705

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History of Changes for Study: NCT00320775

Safety and Tolerability of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

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2	○	○	October 3, 2006	Outcome Measures, Conditions, Study Status, Eligibility, Study Description and Study Identification

Version	A	B	Submitted Date	Changes
3	○	○	July 25, 2007	Recruitment Status, Study Status and Contacts/Locations
4	○	○	January 23, 2009	Recruitment Status, Study Status, Study Design, Arms and Interventions, Contacts/Locations, Outcome Measures, Oversight, Sponsor/Collaborators and Study Identification
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6	○	○	January 26, 2010	Study Status
7	○	○	January 25, 2011	Study Status
8	○	○	June 8, 2011	Study Status
9	○	○	March 16, 2015	Sponsor/Collaborators, Study Status and References

Compare

Comparison Format: ◉ Merged ○ Side-by-Side

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Study NCT00320775

Submitted Date: April 28, 2006 (v1)

Study Identification

Unique Protocol ID: VGFT-OD-0502

Brief Title: Safety and Tolerability of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Official Title: An Exploratory Study of the Safety, Tolerability and Biological Effect of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification:	April 2006
Overall Status:	Recruiting
Study Start:	June 2005
Primary Completion:	
Study Completion:	June 2006
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Last Update Submitted that Met QC Criteria:	April 28, 2006
Last Update Posted:	May 3, 2006 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	

Study Description

Brief Summary:	To assess the ocular and systemic safety and tolerability of a single intravitreal injection of VEGF Trap in patients with subfoveal choroidal neovascularization (CNV) due to AMD.
Detailed Description:	This study consists of three parts, Part A, Part B and Part C.

Conditions

Conditions: Neovascular Age-Related Macular Degeneration

Keywords:

Study Design

Study Type: Interventional

Primary Purpose: Treatment

Study Phase: Phase 1

Interventional Study Model:

Number of Arms:

Masking: (masked roles unspecified)

Allocation: N/A

Enrollment: 96

Arms and Interventions

Intervention Details:

Drug: VEGF Trap

Outcome Measures

Primary Outcome Measures:

1. Safety and tolerability, bioeffect

Secondary Outcome Measures:

2. The effect of VEGF Trap administration on: excess central retinal/lesion thickness, best-corrected ETDRS visual acuity, extent of CNV leakage, anti-VEGF Trap antibodies in the systemic circulation, plasma levels of VEGF Trap

Eligibility

Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Subfoveal CNV secondary to AMD.
- Central retinal/lesion thickness ≥ 250µm as measured by OCT.
- ETDRS best-corrected visual acuity of:
  - 20/40 (73 letters) or worse
- Clear ocular media and clear lens(es) to permit good quality stereoscopic fundus photography.

Exclusion Criteria:

- Prior treatment with VEGF Trap, bevacizumab or ranibizumab.
- Any investigational agent within 12 weeks of Visit 2 (Day 1).
- Presence of other causes of CNV.
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Contacts/Locations

Central Contact: Regeneron
Email: VEGF.Trap@regeneron.com

Locations: **United States, Arizona**

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[Recruiting]

Nashville, Tennessee, United States, 37203

United States, Wisconsin

[Recruiting]

Madison, Wisconsin, United States, 53705

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ClinicalTrials.gov archive

History of Changes for Study: NCT00320775

Safety and Tolerability Study of Intravitreal VEGF-Trap Administration in Patients With Neovascular AMD

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Study Record Versions

Version	A	B	Submitted Date	Changes
1	○	○	April 28, 2006	None (earliest Version on record)
2	○	○	October 3, 2006	Outcome Measures, Conditions, Study Status, Eligibility, Study Description and Study Identification
3	○	○	July 25, 2007	Recruitment Status, Study Status and Contacts/Locations

Version	A	B	Submitted Date	Changes
4	○	○	January 23, 2009	Recruitment Status, Study Status, Study Design, Arms and Interventions, Contacts/Locations, Outcome Measures, Oversight, Sponsor/Collaborators and Study Identification
5	○	○	April 29, 2009	Study Status, Arms and Interventions, Study Description and Sponsor/Collaborators
6	○	○	January 26, 2010	Study Status
7	○	○	January 25, 2011	Study Status
8	○	○	June 8, 2011	Study Status
9	◉	◉	March 16, 2015	Sponsor/Collaborators, Study Status and References

Compare

Comparison Format: ◉ Merged ○ Side-by-Side

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Study NCT00320775

Submitted Date: March 16, 2015 (v9)

Study Identification

Unique Protocol ID: VGFT-OD-0502

Brief Title: Safety and Tolerability Study of Intravitreal VEGF-Trap Administration in Patients With Neovascular AMD

Official Title: An Exploratory Study of the Safety, Tolerability and Biological Effect of Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification: March 2015

Overall Status:	Completed
Study Start:	June 2005
Primary Completion:	June 2008 [Actual]
Study Completion:	August 2008 [Actual]
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Last Update Submitted that Met QC Criteria:	March 16, 2015
Last Update Posted:	March 18, 2015 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	Sponsor
Collaborators:	Bayer

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	No

Study Description

Brief Summary:	The purpose of this trial is to assess the ocular and systemic safety and tolerability of a single intravitreal injection of VEGF Trap in patients with subfoveal choroidal neovascularization (CNV) due to AMD.
Detailed Description:	This study consists of three parts, Part A, Part B and Part C. Part A is a dose escalation. Part B was terminated early. The (one) subject who received Macugen is not discussed in this website. Part C had

subjects receive one of two doses of VEGF Trap (0.15 mg or 4.0 mg).

This is the first study in which human subjects received intravitreal injections of VEGF Trap in a study eye.

Conditions

Conditions: Macular Degeneration

Keywords: Neovascular Age-Related Macular Degeneration

Study Design

Study Type: Interventional

Primary Purpose: Treatment

Study Phase: Phase 1

Interventional Study Model: Parallel Assignment

Number of Arms: 3

Masking: None (Open Label)

Allocation: Randomized

Enrollment: 51 [Actual]

Arms and Interventions

Arms	Assigned Interventions

Arms	Assigned Interventions
Experimental: Part A Part A: An open label study in which six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. The total volume of each injection will be 100 µL. Enrollment in new dose levels will not begin until all patients in the preceding dose level have completed Visit 5 (Day 15).	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

Arms	Assigned Interventions
Active Comparator: Part B Part B: A controlled, prospective, randomized, double-masked study in which up to 30 subjects meeting eligibility criteria will be randomly assigned in a 1:1 ratio to receive a single ITV injection of2.0 mg/eye VEGF Trap (or the MTD if reached prior to 2.0 mg) followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Enrollment into Part B will begin 2 weeks after the last subject to receive the 2.0 mg/eye dose in Part A has been observed for 15 days and it has been determined that the safety profile of VEGF Trap at this dose level is adequate to support expansion of dosing at this dose level. The dose of pegaptanib sodium will be 0.3 mg, according to the package insert.	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

Arms	Assigned Interventions
Active Comparator: Part C Part C: A controlled, prospective, randomized, double-masked study in which approximately 30 subjects meeting eligibility criteria will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. Initiation of Part C is contingent upon the 4.0 mg dose being adequately tolerated in Part A.	Drug: VEGF Trap Part A: Six successive cohorts of 3-6 patients each with neovascular AMD will receive a single intravitreal (ITV) injection of 0.05, 0.15, 0.5, 1.0, 2.0, or 4.0 mg of VEGF Trap into the study eye. Part B: Up to 30 subjects will be randomly assigned in a 1:1 ratio to receive a single of 2.0 mg/eye VEGF Trap followed by 1 sham injection six weeks later, or an initial dose of 0.3 mg pegaptanib sodium into the study eye, followed by a second dose six weeks later. Part C: Approximately 30 subjects will be randomly assigned in a 1:1 ratio to receive up to two ITV injections of either 0.15 or 4.0 mg/eye VEGF Trap. After completion of Visit 8 (Day 57), patients from all parts of the study, may be eligible to continue in Open-label Extension and will receive 4.0 mg of VEGF Trap. Other Names: • Aflibercept

Outcome Measures

Primary Outcome Measures:

1. Safety and tolerability, bioeffect
   From baseline to Day 43

Secondary Outcome Measures:

2. The effect of VEGF Trap administration on excess central retinal/lesion thickness
   From baseline to Day 43
3. Best-corrected Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuity
   From baseline to Day 43
4. Extent of CNV leakage

From baseline to Day 43

5. Anti-VEGF Trap antibodies in the systemic circulation
   From baseline to Day 43
6. Plasma levels of VEGF Trap
   From baseliene to Day 43

Eligibility

Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Subfoveal CNV secondary to AMD.
- Central retinal/lesion thickness ≥ 250µm as measured by optical coherence tomography (OCT).
- ETDRS best-corrected visual acuity of:
  - 20/40 (73 letters) or worse
- Clear ocular media and clear lens(es) to permit good quality stereoscopic fundus photography.

Exclusion Criteria:

- Prior treatment with VEGF Trap, bevacizumab or ranibizumab.
- Any investigational agent within 12 weeks of Visit 2 (Day 1).
- Presence of other causes of CNV.
- Active ocular infection.

Contacts/Locations

Study Officials: Avner Ingerman, MD
Study Director
Regeneron Pharmaceuticals

Locations: **United States, Arizona**

Retina Centers, PC
Tuscon, Arizona, United States, 85704

United States, California

Loma Linda University Health Care
Loma Linda, California, United States, 92354

United States, Illinois

University of Chicago
Chicago, Illinois, United States, 60637

United States, Maryland

Johns Hopkins Hospital School of Medicine
Baltimore, Maryland, United States, 21287

United States, North Carolina

Charlotte Eye, Ear, Nose & Throat Asssociates
Charlotte, North Carolina, United States, 28120

United States, Oklahoma

Dean A. McGee Eye Institute
Oklahoma City, Oklahoma, United States, 73104

United States, Pennsylvania

Retina Diagnostic and Treatment Assoc., LLC
Philadelphia, Pennsylvania, United States, 19107

United States, Tennessee

Retina-Vitreous Associates, P.C.
Nashville, Tennessee, United States, 37203

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References

Citations: **[Study Results]** Nguyen QD, Campochiaro PA, Shah SM, Browning DJ, Hudson HL, Sonkin PL, Hariprasad SM, Kaiser PK, Slakter J, Haller JA, Do DV, Mieler W, Chu K, Ingerman A, Vitti R, Berliner AJ, Cedarbaum J; Clear-It 1 Investigators. Evaluation of very high- and very low-dose intravitreal aflibercept in patients with neovascular age-related macular degeneration. J Ocul Pharmacol Ther. 2012 Dec;28(6):581-8. doi: 10.1089/jop.2011.0261. Epub 2012 Jul 9. PubMed 22775078

[Study Results] Do DV, Schmidt-Erfurth U, Gonzalez VH, Gordon CM, Tolentino M, Berliner AJ, Vitti R, Rückert R, Sandbrink R, Stein D, Yang K, Beckmann K, Heier JS. The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema. Ophthalmology. 2011 Sep;118(9):1819-26. doi: 10.1016/j.ophtha.2011.02.018. Epub 2011 May 5. PubMed 21546089

Links: URL: http://www.ncbi.nlm.nih.gov/pubmed/22775078
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History of Changes for Study: NCT00320788

Safety and Efficacy of Repeated Intravitreal Administration of VEGF-Trap in Patients With Wet AMD

Latest version (submitted January 27, 2012) on ClinicalTrials.gov

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3	○	○	October 3, 2006	Conditions, Study Description, Contacts/Locations, Eligibility, Outcome Measures, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
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5	○	○	January 23, 2009	Recruitment Status, Study Status, Arms and Interventions, Study Design, Contacts/Locations, Outcome Measures, Oversight, Sponsor/Collaborators and Study Identification
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7	○	○	November 30, 2010	Study Status
8	○	○	April 20, 2011	Study Status
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Compare

Comparison Format: ◉ Merged ○ Side-by-Side

Scroll up to access the controls

Study NCT00320788

Submitted Date: December 1, 2011 (v9)

Study Identification

Unique Protocol ID: VGFT-OD-0508

Brief Title: Safety and Efficacy of Repeated Intravitreal Administration of VEGF-Trap in Patients With Wet AMD

Official Title: A Randomized, Controlled Study of the Safety, Tolerability and Biological Effect of Repeated Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification:	December 2011
Overall Status:	Completed
Study Start:	April 2006
Primary Completion:	July 2007 [Actual]
Study Completion:	June 2008 [Actual]
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Certification/Extension First Submitted:	November 30, 2010
Certification/Extension First Submitted that Met QC Criteria:	November 30, 2010
Certification/Extension First Posted:	December 2, 2010 [Estimate]
Last Update Submitted that Met QC Criteria:	December 1, 2011
Last Update Posted:	December 8, 2011 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	Sponsor
Collaborators:	Bayer

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	No

Study Description

Brief Summary: This study examines the effect of intravitreally administered VEGF Trap in patients with wet AMD.

The purpose of this trial is to assess the ocular and systemic safety and tolerability of repeated intravitreal doses of VEGF Trap in patients with subfoveal choroidal neovascularization (CNV) due to AMD.

Detailed Description: This is a double masked, prospective, randomized study in which five groups of approximately 30 patients meeting the eligibility criteria will be randomly assigned in a balanced ratio to receive a series of intravitreal (ITV) injections of VEGF Trap into the study eye at 4- or 12 -week intervals over a 12-week period.

After Week 12, patients will be evaluated every 4 weeks. Patients will remain on study or may be eligible to enter a long-term extension study, in which they will continue to receive VEGF Trap.

Conditions

Conditions:	Macular Degeneration
Keywords:	Neovascular Age-Related Macular Degeneration

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 2
Interventional Study Model:	Parallel Assignment
Number of Arms:	1
Masking:	Triple (Participant, Care Provider, Investigator)
Allocation:	Randomized

Enrollment: 159 [Actual]

Arms and Interventions

Arms	Assigned Interventions
1 This is a double masked, prospective, randomized study in which five groups of approximately 30 patients meeting the eligibility criteria will be randomly assigned in a balanced ratio to receive a series of ITV injections of VEGF Trap into the study eye at 4- or 12-week intervals over a 12-week period. Beginning with Week 16, all patients will be evaluated every 4 weeks for continued dosing for up to one year. The fellow eye may also be eligible for treatment with VEGF Trap beginning at this	Drug: VEGF Trap Subjects in Group A will receive 2 ITV injections of 0.5 mg VEGF Trap at 12-week intervals; Group B subjects will receive 4 ITV injections of 0.5 mg VEGF Trap at 4-week intervals; Group C subjects will receive 2 injections of 2.0 mg VEGF Trap at 12-week intervals; Group D subjects will receive 4 injections of 2.0 mg VEGF Trap at 4-week intervals, and Group E subjects will receive 2 injections of 4.0 mg VEGF Trap at 12-week intervals. Other Names: • Aflibercept

Outcome Measures

Primary Outcome Measures:

1. Safety, biological effect (optical coherence tomography [OCT], fluorescein angiography, visual acuity)
   From baseline to week 12

Secondary Outcome Measures:

2. Pharmacokinetics, immunogenicity, quality of life
   From baseline to week 12

Eligibility

Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Subfoveal CNV secondary to AMD.
- Central retinal (including lesion) thickness ≥ 300 µm as measured by OCT.
- Early Treatment of Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity of 73 letters to 34 letters.

Exclusion Criteria:

- History of any vitreous hemorrhage within 4 weeks prior to Day 1.
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- Significant subfoveal atrophy or scarring.
- Prior treatment with the following in the study eye:
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Contacts/Locations

Study Officials: Avner Ingerman, MD
Study Director

Regeneron Pharmaceuticals

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History of Changes for Study: NCT00320788

Safety and Efficacy of Repeated Intravitreal Administration of VEGF-Trap in Patients With Wet AMD

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Compare

Comparison Format: ◉ Merged ○ Side-by-Side

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Study NCT00320788

Submitted Date: April 20, 2011 (v8)

Study Identification

Unique Protocol ID: VGFT-OD-0508

Brief Title: Safety and Efficacy of Repeated Intravitreal Administration of VEGF-Trap in Patients With Wet AMD

Official Title: A Randomized, Controlled Study of the Safety, Tolerability and Biological Effect of Repeated Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification:	April 2011
Overall Status:	Completed
Study Start:	April 2006
Primary Completion:	July 2007 [Actual]
Study Completion:	June 2008 [Actual]
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Certification/Extension First Submitted:	November 30, 2010
Certification/Extension First Submitted that Met QC Criteria:	November 30, 2010
Certification/Extension First Posted:	December 2, 2010 [Estimate]
Last Update Submitted that Met QC Criteria:	April 20, 2011
Last Update Posted:	April 28, 2011 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	No

Study Description

Brief Summary: This study examines the effect of intravitreally administered VEGF Trap in patients with wet AMD.

The purpose of this trial is to assess the ocular and systemic safety and tolerability of repeated intravitreal doses of VEGF Trap in patients with subfoveal choroidal neovascularization (CNV) due to AMD.

Detailed Description: This is a double masked, prospective, randomized study in which five groups of approximately 30 patients meeting the eligibility criteria will be randomly assigned in a balanced ratio to receive a series of intravitreal (ITV) injections of VEGF Trap into the study eye at 4- or 12 -week intervals over a 12-week period.

After Week 12, patients will be evaluated every 4 weeks. Patients will remain on study or may be eligible to enter a long-term extension study, in which they will continue to receive VEGF Trap.

Conditions

Conditions:	Macular Degeneration
Keywords:	Neovascular Age-Related Macular Degeneration

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 2
Interventional Study Model:	Parallel Assignment
Number of Arms:	1
Masking:	Triple (Participant, Care Provider, Investigator)
Allocation:	Randomized

Enrollment: 159 [Actual]

Arms and Interventions

Arms	Assigned Interventions
1 This is a double masked, prospective, randomized study in which five groups of approximately 30 patients meeting the eligibility criteria will be randomly assigned in a balanced ratio to receive a series of ITV injections of VEGF Trap into the study eye at 4- or 12-week intervals over a 12-week period. Beginning with Week 16, all patients will be evaluated every 4 weeks for continued dosing for up to one year. The fellow eye may also be eligible for treatment with VEGF Trap beginning at this	Drug: VEGF Trap Subjects in Group A will receive 2 ITV injections of 0.5 mg VEGF Trap at 12-week intervals; Group B subjects will receive 4 ITV injections of 0.5 mg VEGF Trap at 4-week intervals; Group C subjects will receive 2 injections of 2.0 mg VEGF Trap at 12-week intervals; Group D subjects will receive 4 injections of 2.0 mg VEGF Trap at 4-week intervals, and Group E subjects will receive 2 injections of 4.0 mg VEGF Trap at 12-week intervals. Other Names: • Aflibercept

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Primary Outcome Measures:

1. Safety, biological effect (optical coherence tomography [OCT], fluorescein angiography, visual acuity)
   From baseline to week 12

Secondary Outcome Measures:

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Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

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U.S. National Library of Medicine

ClinicalTrials.gov archive

History of Changes for Study: NCT00320788

Safety and Efficacy of Repeated Intravitreal Administration of VEGF-Trap in Patients With Wet AMD

Latest version (submitted January 27, 2012) on ClinicalTrials.gov

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Compare

Comparison Format: ◉ Merged ○ Side-by-Side

Scroll up to access the controls

Study NCT00320788

Submitted Date: November 30, 2010 (v7)

Study Identification

Unique Protocol ID: VGFT-OD-0508

Brief Title: Safety and Efficacy of Repeated Intravitreal Administration of VEGF-Trap in Patients With Wet AMD

Official Title: A Randomized, Controlled Study of the Safety, Tolerability and Biological Effect of Repeated Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification:	November 2010
Overall Status:	Completed
Study Start:	April 2006
Primary Completion:	June 2008 [Actual]
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First Submitted:	April 28, 2006
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Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	
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Data Monitoring:	No

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Conditions:	Macular Degeneration
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Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	Phase 2
Interventional Study Model:	Parallel Assignment
Number of Arms:	1
Masking:	Triple (Participant, Care Provider, Investigator)
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Enrollment: 159 [Actual]

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5	○	○	January 23, 2009	Recruitment Status, Study Status, Arms and Interventions, Study Design, Contacts/Locations, Outcome Measures, Oversight, Sponsor/Collaborators and Study Identification
6	◉	◉	April 28, 2009	Study Status
7	○	○	November 30, 2010	Study Status
8	○	○	April 20, 2011	Study Status
9	○	○	December 1, 2011	Sponsor/Collaborators, Study Status
10	○	○	January 27, 2012	Arms and Interventions, Study Status, Outcome Measures, More Information, Reported Adverse Events, Baseline Characteristics, Participant Flow, References, Contacts/Locations, Eligibility, Study Description and Study Identification

Compare

Comparison Format: ◉ Merged ○ Side-by-Side

Scroll up to access the controls

Study NCT00320788

Submitted Date: April 28, 2009 (v6)

Study Identification

Unique Protocol ID: VGFT-OD-0508

Brief Title: Safety and Efficacy of Repeated Intravitreal Administration of VEGF-Trap in Patients With Wet AMD

Official Title: A Randomized, Controlled Study of the Safety, Tolerability and Biological Effect of Repeated Intravitreal Administration of VEGF Trap in Patients With Neovascular Age-Related Macular Degeneration

Secondary IDs:

Study Status

Record Verification:	April 2009
Overall Status:	Completed
Study Start:	April 2006
Primary Completion:	June 2008 [Actual]
Study Completion:	August 2008 [Actual]
First Submitted:	April 28, 2006
First Submitted that Met QC Criteria:	April 28, 2006
First Posted:	May 3, 2006 [Estimate]
Last Update Submitted that Met QC Criteria:	April 28, 2009
Last Update Posted:	April 29, 2009 [Estimate]

Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	No

Study Description

Brief Summary:	This study examines the effect of intravitreally administered VEGF Trap in patients with wet AMD.

The purpose of this trial is to assess the ocular and systemic safety and tolerability of repeated intravitreal doses of VEGF Trap in patients with subfoveal choroidal neovascularization (CNV) due to AMD.

Detailed Description: This is a double masked, prospective, randomized study in which five groups of approximately 30 patients meeting the eligibility criteria will be randomly assigned in a balanced ratio to receive a series of intravitreal (ITV) injections of VEGF Trap into the study eye at 4- or 12 -week intervals over a 12-week period.

After Week 12, patients will be evaluated every 4 weeks. Patients will remain on study or may be eligible to enter a long-term extension study, in which they will continue to receive VEGF Trap.

Conditions

Conditions: Macular Degeneration

Keywords: Neovascular Age-Related Macular Degeneration

Study Design

Study Type: Interventional

Primary Purpose: Treatment

Study Phase: Phase 2

Interventional Study Model: Parallel Assignment

Number of Arms: 1

Masking: Triple (Participant, Care Provider, Investigator)

Allocation: Randomized

Enrollment: 159 [Actual]

Arms and Interventions

Arms	Assigned Interventions

Arms	Assigned Interventions
1 This is a double masked, prospective, randomized study in which five groups of approximately 30 patients meeting the eligibility criteria will be randomly assigned in a balanced ratio to receive a series of ITV injections of VEGF Trap into the study eye at 4- or 12-week intervals over a 12-week period. Beginning with Week 16, all patients will be evaluated every 4 weeks for continued dosing for up to one year. The fellow eye may also be eligible for treatment with VEGF Trap beginning at this	Drug: VEGF Trap Subjects in Group A will receive 2 ITV injections of 0.5 mg VEGF Trap at 12-week intervals; Group B subjects will receive 4 ITV injections of 0.5 mg VEGF Trap at 4-week intervals; Group C subjects will receive 2 injections of 2.0 mg VEGF Trap at 12-week intervals; Group D subjects will receive 4 injections of 2.0 mg VEGF Trap at 4-week intervals, and Group E subjects will receive 2 injections of 4.0 mg VEGF Trap at 12-week intervals. Other Names: • Aflibercept

Outcome Measures

Primary Outcome Measures:

1. Safety, biological effect (optical coherence tomography [OCT], fluorescein angiography, visual acuity)
   From baseline to week 12

Secondary Outcome Measures:

2. Pharmacokinetics, immunogenicity, quality of life
   From baseline to week 12

Eligibility

Minimum Age: 50 Years

Maximum Age:

Sex: All

Gender Based:

Accepts Healthy Volunteers: No

Criteria: Inclusion Criteria:

- Subfoveal CNV secondary to AMD.
- Central retinal (including lesion) thickness ≥ 300 µm as measured by OCT.
- Early Treatment of Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity of 73 letters to 34 letters.

Exclusion Criteria:

- History of any vitreous hemorrhage within 4 weeks prior to Day 1.
- Aphakia.
- Significant subfoveal atrophy or scarring.
- Prior treatment with the following in the study eye:
  - Subfoveal thermal laser therapy.
  - Submacular surgery or other surgical intervention for the treatment of AMD.
  - Extrafoveal laser coagulation treatment within 12 weeks prior to Day 1.
  - Photodynamic therapy (PDT) within 12 weeks prior to Visit 2 (Day 1).
  - Pegaptanib sodium (Macugen) within 8 weeks of Visit 2 (Day 1).
  - Juxtascleral steroids or anecortave acetate within 24 weeks (6 months) prior to Visit 2 (Day 1).
  - Intravitreal administration of triamcinolone acetonide or other steroids within 24 weeks prior to Visit 2 (Day 1), unless no visible residue of drug substance can be seen in the vitreous cavity using indirect ophthalmoscopy.
  - Prior systemic or intravitreal treatment with VEGF Trap, ranibizumab (Lucentis) or bevacizumab (Avastin).
- Presence of any other condition or laboratory abnormality, which, in the opinion of the Investigator, would interfere with the assessment of disease status/progression or jeopardize the patient's appropriate participation in this Phase II study.

Contacts/Locations

Study Officials: Avner Ingerman, MD
Study Director
Regeneron Pharmaceuticals

Locations: **United States, Arizona**

Associated Retina Consultants

Phoenix, Arizona, United States, 85020

Retina Centers, PC
Tucson, Arizona, United States, 85704

United States, California

Retina Vitreous Associates Medical Group
Beverly Hills, California, United States, 90211

Loma Linda University Health Care
Loma Linda, California, United States, 92354

United States, Georgia

Southeast Retina Center
Augusta, Georgia, United States, 30909

United States, Illinois

University of Chicago
Chicago, Illinois, United States, 60637

United States, Indiana

Midwest Eye Institute
Indianapolis, Indiana, United States, 46280

United States, Maryland

Johns Hopkins Hospital School of Medicine
Baltimore, Maryland, United States, 21287

United States, Massachusetts

Ophthalmic Consultants of Boston
Boston, Massachusetts, United States, 02114

New England Retina Consultants PC
West Springfield, Massachusetts, United States, 10189

United States, North Carolina

Charlotte Eye, Ear, Nose & Throat Asssociates

Charlotte, North Carolina, United States, 28210

United States, Oklahoma

Dean A. McGee Eye Institute
Oklahoma City, Oklahoma, United States, 73104

United States, Oregon

Retina Northwest PC
Portland, Oregon, United States, 97210

United States, Pennsylvania

Retina Diagnostic and Treatment Assoc., LLC
Philadelphia, Pennsylvania, United States, 19107

United States, South Dakota

Black Hills Regional Eye Institute
Rapid City, South Dakota, United States, 57701

United States, Tennessee

Retina-Vitreous Associates, P.C.
Nashville, Tennessee, United States, 37203

United States, Texas

Vitreoretinal Consultants Scurlock Tower Texas Medical Center
Houston, Texas, United States, 77030

Medical Center Ophthamology
San Antonio, Texas, United States, 78240

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Citations:

Links:

Available IPD/Information:

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U.S. National Library of Medicine

ClinicalTrials.gov archive

History of Changes for Study: NCT00320788

Safety and Efficacy of Repeated Intravitreal Administration of VEGF-Trap in Patients With Wet AMD

Latest version (submitted January 27, 2012) on ClinicalTrials.gov

- A study version is represented by a row in the table.
- Select two study versions to compare. One each from columns A and B.
- Choose either the "Merged" or "Side-by-Side" comparison format to specify how the two study versions are to be displayed. The Side-by-Side format only applies to the Protocol section of the study.
- Click "Compare" to do the comparison and show the differences.
- Select a version's Submitted Date link to see a rendering of the study for that version.
- The yellow A/B choices in the table indicate the study versions currently compared below. A yellow table row indicates the study version currently being viewed.
- Hover over the "Recruitment Status" to see how the study's recruitment status changed.
- Study edits or deletions are displayed in ~~red~~.
- Study additions are displayed in *green*.

Study Record Versions

Version	A	B	Submitted Date	Changes
1	○	○	April 28, 2006	None (earliest Version on record)
2	○	○	August 1, 2006	Contacts/Locations and Study Status
3	○	○	October 3, 2006	Conditions, Study Description, Contacts/Locations, Eligibility, Outcome Measures, Study Status and Study Identification

Version	A	B	Submitted Date	Changes
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Study NCT00320788

Submitted Date: January 23, 2009 (v5)

Study Identification

Unique Protocol ID: VGFT-OD-0508

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Sponsor/Collaborators

Sponsor:	Regeneron Pharmaceuticals
Responsible Party:	
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
Data Monitoring:	No

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