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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/943,490	11/17/2015	Robert L. VITTI	REGN-020	1006

96387 7590 05/08/2017

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

EXAMINER
SPECTOR, LORRAINE

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
05/08/2017	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

ALVOTECH 1082
Alvotech v. Regeneron
PGR2025-00085

Notice of Abandonment	Application No.	Applicant(s)
	14/943,490	VITTI ET AL.
	Examiner	Art Unit
	/LORRAINE SPECTOR/	1647

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

This application is abandoned in view of:

1. ☒ Applicant's failure to timely file a proper reply to the Office letter mailed on *27 September 2016*.
   (a) ☐ A reply was received on _____ (with a Certificate of Mailing or Transmission dated _____), which is after the expiration of the period for reply (including a total extension of time of _____ month(s)) which expired on _____.
   (b) ☐ A proposed reply was received on _____, but it does not constitute a proper reply under 37 CFR 1.113 to the final rejection.
   (A proper reply under 37 CFR 1.113 to a final rejection consists only of: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) if this is utility or plant application, a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. Note that RCEs are not permitted in design applications.)
   (c) ☐ A reply was received on _____ but it does not constitute a proper reply, or a bona fide attempt at a proper reply, to the non-final rejection. See 37 CFR 1.85(a) and 1.111. (See explanation in box 7 below).
   (d) ☐ No reply has been received.

2. ☐ Applicant's failure to timely pay the required issue fee and publication fee, if applicable, within the statutory period of three months from the mailing date of the Notice of Allowance (PTOL-85).
   (a) ☐ The issue fee and publication fee, if applicable, was received on _____ (with a Certificate of Mailing or Transmission dated _____), which is after the expiration of the statutory period for payment of the issue fee (and publication fee) set in the Notice of Allowance (PTOL-85).
   (b) ☐ The submitted fee of $_____ is insufficient. A balance of $_____ is due.
   The issue fee required by 37 CFR 1.18 is $_____. The publication fee, if required by 37 CFR 1.18(d), is $_____.
   (c) ☐ The issue fee and publication fee, if applicable, has not been received.

3. ☐ Applicant's failure to timely file corrected drawings as required by, and within the three-month period set in, the Notice of Allowability (PTO-37).
   (a) ☐ Proposed corrected drawings were received on _____ (with a Certificate of Mailing or Transmission dated _____), which is after the expiration of the period for reply.
   (b) ☐ No corrected drawings have been received.

4. ☐ The letter of express abandonment which is signed by the attorney or agent of record or other party authorized under 37 CFR 1.33(b). See 37 CFR 1.138(b).

5. ☐ The letter of express abandonment which is signed by an attorney or agent (acting in a representative capacity under 37 CFR 1.34) upon the filing of a continuing application.

6. ☐ The decision by the Board of Patent Appeals and Interference rendered on _____ and because the period for seeking court review of the decision has expired and there are no allowed claims.

7. ☐ The reason(s) below:

/Lorraine Spector/
Primary Examiner
Art Unit: 1647

Petitions to revive under 37 CFR 1.137, or requests to withdraw the holding of abandonment under 37 CFR 1.181, should be promptly filed to minimize any negative effects on patent term.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	14/943,490
				Filing Date	November 17, 2015
				First Named Inventor	Vitti, Robert L.
				Art Unit	1647
				Examiner Name	SPECTOR, LORRAINE
Sheet	1	of	1	Attorney Docket Number	REGN-020

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	**8268314**	2012-09-18	BAEHNER et al.	
	2	**8703130**	2014-04-22	BAEHNER 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	**2014/0017244**	2014-01-16	DUERR 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	KOENIG et al., "Deep Sequencing-guided Design of a High Affinity Dual Specificity Antibody to Target Two Angiogenic Factors in Neovascular Age-related Macular Degeneration" J. Biol. Chem. (September 4, 2015) 290(36):21773-21786	
	2	OLINER et al., "AMG 386, a Selective Angiopoietin ½-Neutralizing Peptibody, Inhibits Angiogenesis in Models of Ocular Neovascular Diseases" Investigative O[t\hthalmology & Visual Science (April 2012) 53(4):2170-2180	
	3	RENNEL et al., "A Human Neutralizing Antibody Specific to Ang-2 Inhibits Ocular Angiogenesis" Microcirculation, The Official Journal of the Microcirculatory Society, Inc. (2011) 18:598-607	
	4	RUBIO et al., "Ocular Angiogenesis: Vascular Endothelial Growth Factor and Other Factors" Dev Ophthalmol. Basel,Karger (2016) 55:28-37	
	5	TUUMINEN et al., "Increased intravitreal angiopoietin-2 levels in patients with retinal vein occlusion" Acta Ophthalmologica (2014) e164-e165	
	6	YOU et al., "Effects of laser photocoagulation on serum angiopoietin-1, angiopoietin-2, angiopoietin-1/angiopoietin-2 ratio, and soluble angiopoietin receptor Tie-2 levels in type 2 diabetic patients with proliferative diabetic retinopathy" Int J. Ophthalmol (August 18, 2014) 7(4):648-653	

Examiner Signature		Date Considered	

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

Electronic Patent Application Fee Transmittal					
Application Number:	14943490				
Filing Date:	17-Nov-2015				
Title of Invention:	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES				
First Named Inventor/Applicant Name:	Robert L. VITTI				
Filer:	Karl Bozicevic/Kimberly Zuehlke				
Attorney Docket Number:	REGN-020				
Filed as Large Entity					
Filing Fees for Utility under 35 USC 111(a)					
Description		**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:					
Pages:					
Claims:					
Miscellaneous-Filing:					
Petition:					
Patent-Appeals-and-Interference:					
Post-Allowance-and-Post-Issuance:					
Extension-of-Time:					

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt	
EFS ID:	27279475
Application Number:	14943490
International Application Number:	
Confirmation Number:	1006
Title of Invention:	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES
First Named Inventor/Applicant Name:	Robert L. VITTI
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-020
Receipt Date:	21-OCT-2016
Filing Date:	17-NOV-2015
Time Stamp:	11:12:57
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$180
RAM confirmation Number	102116INTEFSW11134500
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	REGN-020_2016-10-21_Supp_IDS_trans.pdf	52846 52c9f2fbc88f0cefadc1972db176875706b745cc	no	2
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	REGN-020_2016-10-21_supp_IDS.pdf	27173 c04aed71e42ad1e26a6afc1e6e86f430b9c0429e	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Non Patent Literature	Koenig2015.pdf	3767574 e827c761867770476cb3979d197e2ee531ff9089	no	15
Warnings:					
Information:					
4	Non Patent Literature	Rennel2011.pdf	830357 775e22c304a35660af69f4cbe406b4e957c5b714	no	11
Warnings:					
Information:					
5	Non Patent Literature	Rubio2016.pdf	130087 13161fe5e1749a721735eba3e6cdb6a0d832b987	no	10
Warnings:					
Information:					
6	Non Patent Literature	You2013.pdf	344327 ff47b776e4029c9595431ccbd27edf8079d03d74	no	6
Warnings:					

Information:					
7	Non Patent Literature	Oliner_2012.pdf	1607976 e2a34f6f32e4b53e528aff760489ad83e0c59a54	no	11
Warnings:					
Information:					
8	Non Patent Literature	Tuuminen_2014.pdf	153839 5d0380546571ee82056f0176fb1481a3e8fe03de	no	3
Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	30931 0362457d64cec51c6b0222bb68668a062c5508c9	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	6945110	

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

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronically Filed

INFORMATION DISCLOSURE STATEMENT Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450		
	Attorney Docket	REGN-020
	First Named Inventor	Vitti, Robert L.
	Application Number	14/943,490
	Confirmation No.	1006
	Filing Date	November 17, 2015
	Group Art Unit	1647
	Examiner Name	SPECTOR, LORRAINE
	Title: "*Methods and Formulations for Treating Vascular Eye Diseases*"	

Sir:

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

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

Statements

☒ **No statement**

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

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

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

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

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

Fees

☐ No fee is believed to be due.

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

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-020.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: October 21, 2016

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

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, California 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/943,490	11/17/2015	Robert L. VITTI	REGN-020	1006

96387 7590 09/27/2016

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

EXAMINER
SPECTOR, LORRAINE

ART UNIT	PAPER NUMBER
1647	

NOTIFICATION DATE	DELIVERY MODE
09/27/2016	ELECTRONIC

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

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

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

docket@bozpat.com

Office Action Summary	Application No. 14/943,490	Applicant(s) VITTI ET AL.	
	Examiner /LORRAINE SPECTOR/	Art Unit 1647	AIA (First Inventor to File) Status Yes

-- 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 _____.

☐ 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) *93-122* 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) *93-122* 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 *11/17/15* 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 *3/2/16, 4/20/16*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Specification

The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed.

The title should refer to the active agents being used. Correction is required.

The disclosure is objected to because of the following informalities: the specification refers in several places to "anti-Ang-2-inhibitor". The examiner believes that applicants intend "anti-Ang-2" or "Ang-2 inhibitor".

Appropriate correction is required.

Claim Interpretation

In considering and applying the prior art, the dosage limitations of claims 94-100 and 105-109 are given minimal weight- a recitation of the amount of a drug without any definite recitation of what the drug *is* is practically meaningless.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 93-122 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are drawn to the treatment of vascular eye disease, comprising administration of an anti-Ang-2 inhibitor and a VEGF antagonist. However, the claims either do not specify the nature of either active agent, or specify one but not the other.

At paragraph 69 of the specification (referring to the PG-PUB), it is stated:

> 0069: [0069] Non-limiting examples of categories of Ang-2 inhibitors include small molecule Ang-2 inhibitors, anti-Ang-2 aptamers, peptide-based Ang-2 inhibitors (e.g., "peptibody" molecules), "receptor-bodies" (e.g., engineered molecules comprising the receptor-binding domain of an Ang-2 component), and antibodies or antigen-binding fragments of antibodies that specifically bind human Ang-2. In certain embodiments, the Ang-2 inhibitor is an antibody or antigen binding fragment thereof as disclosed in e.g., US Patent Application Publication No. US20110027286. In certain embodiments, the anti-Ang-2 antibody or antigen binding fragment thereof comprises a heavy chain variable region (HCVR) having an amino acid sequence of SEQ ID NO: 1 and a light chain variable region (LCVR) having an amino acid sequence of SEQ ID NO: 2.

There is no actual written description of any “peptibody”, small molecule, or aptamer. A single species of anti-Ang2 antibody was used in the working examples, see for example paragraph 142.

With further respect to ang-2, the specification and claims also disclose that an anti-ang-2 *inhibitor* can be used. There is no written description of any such inhibitor nor how it would work in the context of the invention.

With respect to VEGF inhibitors, paragraph 22 of the specification states the following:

> VEGF antagonists that may be used in combination with an Ang-2 inhibitor in the compositions and methods of the present invention include anti-VEGF antibodies (e.g., ranibizumab), small molecule VEGF inhibitors (e.g., sunetinib), and VEGF-inhibiting fusion proteins ("VEGF Traps"). An example of

> a VEGF antagonist that may be used in combination with the anti-Ang-2 antibodies in the methods of treatment of the present invention is aflibercept, a VEGF-inhibiting fusion protein (see U.S. Pat. No. 7,087,411).

There is no description of VEGF Traps (VEGF-inhibiting fusion proteins) other than by function, only a single example of a small molecule VEGF inhibitor.

Without knowing what the active agents are, the written description fails to support the breadth of the claims.

> The following is a quotation of 35 U.S.C. 112(b):
> (b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

> The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 93-122 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

The claims refer in several places to "anti-Ang-2-inhibitor". The examiner believes that applicants intend "anti-Ang-2" or "Ang-2 inhibitor", as the double negative does not make sense in the context of the invention.

Claim 118 is further indefinite because it is not clear whether or not "(VEGF Trap)" is a limitation.

Prior Art

Claim Rejections - 35 USC § 102

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the

statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a)(2) the claimed invention was described in a patent issued under section 151, or in an application for patent published or deemed published under section 122(b), in which the patent or application, as the case may be, names another inventor and was effectively filed before the effective filing date of the claimed invention.

Claim(s) s 93-100, 105-114 and 118-122 is/are rejected under 35 U.S.C. 102(a)(2) as being anticipated by United States Patent Number 9062105, filed 11/14/2014, (Clube).

Clube teaches and claims A method of treating or reducing the risk of retinopathy in a human, wherein the retinopathy is diabetic retinopathy (DR) or retinopathy of prematurity (ROP), and the active agent is anti-VEGF-A trap; antibody; or antibody fragment (claims 1-3). Also claimed is the method comprising administering an anti-Ang2 or anti-Tie2 ligand to the human simultaneously or sequentially with the anti-VEGF-A ligand (claim 22), where the patient is suffering from type I or type II diabetes, or is elderly (claims 25-27). Also claimed is the method of claim 1, wherein the method comprises administering an anti-Ang2 or anti-Tie2 ligand to the human simultaneously or sequentially with the anti-VEGF-A ligand (claim 24). The anti-Ang2 ligand can be an antibody or fragment, trap, aptamer, of "New Chemical Entity";, see DETX (1415), and may be administered simultaneously of sequentially with the anti-VEGF-A ligand. Note that the Ang2 receptor is known as Tie2.

Treatment of age-related macular degeneration and a variety of other conditions is disclosed at column 366 beginning at line 9, for example.

The use of aflibercept is disclosed at column 369, line 12.

Topical, intraocular and intravitrial administration are disclosed at column 369, beginning at line 13. Also at column 369, administration of multiple doses is disclosed, and the determination of such indicated to be routine in the art; see the two paragraphs beginning at line 17.

Accordingly, the claims are *prima facie* anticipated by Clube.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims the examiner presumes that the subject matter of the various claims was commonly owned as of the effective filing date of the claimed invention(s) absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and effective filing dates of each claim that was not commonly owned as of the effective filing date of the later invention in order for the examiner to consider the applicability of 35 U.S.C. 102(b)(2)(C) for any potential 35 U.S.C. 102(a)(2) prior art against the later invention.

Claims 101-104 is/are rejected under 35 U.S.C. 103 as being unpatentable over Clube, United States Patent Number 9062105.

The teachings of Clube are discussed above. While Clube discloses administration of multiple doses is disclosed, and that the determination of such indicated to be routine in the art, the reference does not disclose the particular dosage schemes of the rejected claims. However, it would have been obvious to the person having ordinary skill in the art at the time the invention was made (Pre-AIA) or effectively filed (AIA) to optimize dosage schemes, which would, as taught by Clube, be routine in the art.

Accordingly, the claims are *prima facie* obvious over Clube.

Claims 115-117 is/are rejected under 35 U.S.C. 103 as being unpatentable over Clube, United States Patent Number 9062105 in view of PG-PUB 2012/0189635 (Thurston).

The teachings of Clube are discussed above. Clube does not disclose an anti-Ang-2 antibody comprising SEQ ID NO: 1 and 2 of the instant application.

Claims 115-117 add limitations as to the amino acid sequences of the anti-Ang-2 antibodies.

At paragraph 10, Thurston discloses an anti-Ang-2 antibody comprising SEQ ID NO: 1 and 2 of the instant application (SEQ ID NO: 18 and 20 of Thurston).

For applicant's convenience, the alignments are reproduced herein:

```
RESULT 2
US-13-417-372-18
; Sequence 18, Application US/13417372
; Publication No. US20120189635A1
; GENERAL INFORMATION
;  APPLICANT: Gavin Thurston
;  APPLICANT:Christopher Daly
;  APPLICANT:Lisa Purcell Ngambo
;  TITLE OF INVENTION: Methods for Treating or Preventing Malaria by
Administering
;  TITLE OF INVENTION:an Antibody that Specifically Binds Angiopoietin-2
(Ang-2)
;  FILE REFERENCE: 6200B
;  CURRENT APPLICATION NUMBER: US/13/417,372
;  CURRENT FILING DATE: 2012-03-12
;  PRIOR APPLICATION NUMBER: 61/229,418
;  PRIOR FILING DATE: 2009-07-29
;  PRIOR APPLICATION NUMBER: 61/295,194
;  PRIOR FILING DATE: 2010-01-15
;  PRIOR APPLICATION NUMBER: 12/843,905
;  PRIOR FILING DATE: 2010-07-27
;  NUMBER OF SEQ ID NOS: 531
;  SOFTWARE: FastSEQ for Windows Version 4.0
; SEQ ID NO 18
;  LENGTH: 122
;  TYPE: PRT
;  ORGANISM: Artificial Sequence
;  FEATURE:
;  OTHER INFORMATION: synthetic
US-13-417-372-18

  Query Match             100.0%;  Score 643;  DB 10;  Length 122;
  Best Local Similarity   100.0%;
  Matches  122;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps
0;
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```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDIHWVRQATGKGLEWVSAIGPAGDTYYP
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Db          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDIHWVRQATGKGLEWVSAIGPAGDTYYP
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Qy         61 GSVKGRFTISRENAKNSLYLQMNSLRAGDTAVYYCARGLITFGGLIAPFDYWGQGTLVTV
120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 GSVKGRFTISRENAKNSLYLQMNSLRAGDTAVYYCARGLITFGGLIAPFDYWGQGTLVTV
120

Qy        121 SS 122
              ||
Db        121 SS 122
```

```
RESULT 3
US-13-417-372-20
; Sequence 20, Application US/13417372
; Publication No. US20120189635A1
; GENERAL INFORMATION
;  APPLICANT: Gavin Thurston
;  APPLICANT:Christopher Daly
;  APPLICANT:Lisa Purcell Ngambo
;  TITLE OF INVENTION: Methods for Treating or Preventing Malaria by
Administering
;  TITLE OF INVENTION:an Antibody that Specifically Binds Angiopoietin-2
(Ang-2)
;  FILE REFERENCE: 6200B
;  CURRENT APPLICATION NUMBER: US/13/417,372
;  CURRENT FILING DATE: 2012-03-12
;  PRIOR APPLICATION NUMBER: 61/229,418
;  PRIOR FILING DATE: 2009-07-29
;  PRIOR APPLICATION NUMBER: 61/295,194
;  PRIOR FILING DATE: 2010-01-15
;  PRIOR APPLICATION NUMBER: 12/843,905
;  PRIOR FILING DATE: 2010-07-27
;  NUMBER OF SEQ ID NOS: 531
;  SOFTWARE: FastSEQ for Windows Version 4.0
; SEQ ID NO 20
;  LENGTH: 107
;  TYPE: PRT
;  ORGANISM: Artificial Sequence
;  FEATURE:
;  OTHER INFORMATION: synthetic
US-13-417-372-20

  Query Match             100.0%;  Score 555;  DB 10;  Length 107;
```

```
  Best Local Similarity   100.0%;
  Matches  107;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps
0;

Qy          1 EIVLTQSPGTLSLSPGERATLSCRASQSVSSTYLAWYQQKPGQAPRLLIYGASSRATGIP
60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EIVLTQSPGTLSLSPGERATLSCRASQSVSSTYLAWYQQKPGQAPRLLIYGASSRATGIP
60

Qy         61 DRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYDNSQTFGQGTKVEIK 107
              |||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 DRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYDNSQTFGQGTKVEIK 107
```

It would have been obvious to the person having ordinary skill in the art at the time the invention was made (Pre-AIA) or effectively filed (AIA) to use the antibody of Thurston in the methods of Clube et al. in view of Clube's teaching of using anti-Ang-2 antibodies, and Thurston's disclosure of anti-Ang-2 antibodies that inhibit Ang-2. Accordingly, the invention as claimed is *prima facie* obvious.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to /LORRAINE SPECTOR/ whose telephone number is (571)272-0893. The examiner can normally be reached on M 8:30-5, Tu-F 9-3, Telework Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joanne Hama can be reached on 571-232-2911. The fax phone 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.

/Lorraine Spector/
Primary Examiner
Art Unit 1647

Notice of References Cited	Application/Control No. 14/943,490	Applicant(s)/Patent Under Reexamination VITTI ET AL.	
	Examiner /LORRAINE SPECTOR/	Art Unit 1647	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-9,062,105 B1	06-2015	Clube; Jasper Rupert	A61K39/3955	1/1
*	B	US-2012/0189635 A1	07-2012	Thurston; Gavin	A61K31/513	424/139.1
	C	US-				
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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BIB DATA SHEET

CONFIRMATION NO. 1006

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
14/943,490	11/17/2015 RULE	514	1647	REGN-020

APPLICANTS

REGENERON PHARMACEUTICALS, INC., Tarrytown, NY

INVENTORS

Robert L. VITTI, Old Tappan, NJ;
Kristine A. ERICKSON, Roxbury, CT;
Karen W. CHU, White Plains, NY;
Stanley J. WIEGAND, Hopewell Junction, NY;
Jingtai CAO, White Plains, NY;
Ivan B. LOBOV, New York, NY;
Saurabh WADHWA, Elmsford, NY;
Kenneth S. GRAHAM, Pleasant Valley, NY;
Daniel DIX, LaGrangeville, NY;

**** CONTINUING DATA ***************************

This appln claims benefit of 62/084,003 11/25/2014
and claims benefit of 62/147,232 04/14/2015

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☐ No Verified and Acknowledged /LORRAINE SPECTOR/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		NJ	4	30	1

ADDRESS

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

TITLE

METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES

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2540	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

BIB (Rev. 05/07).

PTO/SB/08a (01-10)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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

Application Number	14943490
Filing Date	2015-11-17
First Named Inventor	Vitti, Robert L.
Art Unit	
Examiner Name	
Attorney Docket Number	REGN-020

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	2344537	EP		2014-01-29	F. Hoffmann-La Roche AG		

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NON-PATENT LITERATURE DOCUMENTS Remove

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14943490
	Filing Date	2015-11-17
	First Named Inventor	Vitti, Robert L.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-020

	1	Koenig et al. "Deep Sequencing-guided Design of a High Affinity Dual Specificity Antibody to Target Two Angiogenic Factors in Neovascular Age-related Macular Degeneration" Journal of Biological Chemistry, September 4, 2015, Vol. 290, No. 36. pp. 21773-21786.	
	2	Oliner et al. "AMG 386, a Selective Angiopoietin 1/2-Neutralizing Peptibody, Inhibits Angiogenesis in Models of Ocular Neovascular Diseases" Investigative Ophthalmology & Visual Science, April 2012, Vol. 53, No. 4, pp. 2170-2180.	
	3	Rennel et al. "A Human Neutralizing Antibody Specific to Ang-2 Inhibits Ocular Angiogenesis" 2011, Microcirculation, Vol. 18, pp. 598-607.	
	4	Rubio et al. "Ocular Angiogenesis: Vascular Endothelial Growth Factor and Other Factors" Retinal Pharmacotherapeutics, 2016, Vol. 55, pp. 28-37.	
	5	Tuuminen et al. "Increased Intravitreal angiopoietin-2 levels in patients with retinal vein occlusion" 2014, pp. 164-165.	
	6	You et al. "Effects of laser photocoagulation on serum agiopoietin-1, agiopoietin-2, angiopoietin-1/angiopoietin-2 ratio,, and soluble angiopoietin receptor Tie-2 levels in type 2 diabetic patients with proliferative diabetic retinopathy." International Journal of Ophthalmology, Vol. 7, No. 4, August 18, 2014, pp. 648-653.	

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

EXAMINER SIGNATURE

Examiner Signature	/LORRAINE SPECTOR/	Date Considered	09/20/2016

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

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14943490
	Filing Date	2015-11-17
	First Named Inventor	Vitti, Robert L.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-020

CERTIFICATION STATEMENT

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

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

OR

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

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Karl Bozicevic, Reg. No. 28,807/	Date (YYYY-MM-DD)	2016-03-02
Name/Print	Karl Bozicevic	Registration Number	28807

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

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

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

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

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

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

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14943490	VITTI ET AL.
	Examiner	Art Unit
	LORRAINE SPECTOR	1647

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor Name search -PALM ; looked at 22 cases for DP (based on titles)		
EAST- encl.	9/21/2016	LMS
SEQ SEA- in file	9/21/16	LMS

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1	"20160144025" and vegf adj inhibitor	US-PGPUB; USPAT	OR	ON	2016/09/21 09:02
L2	0	"20160144025" and polypoidal adj choridal adj vasculopathy	US-PGPUB; USPAT	OR	ON	2016/09/21 09:11
L3	1	"20160144025" and polypoidal	US-PGPUB; USPAT	OR	ON	2016/09/21 09:11
L4	0	"20160144025" and polyploidal adj choridal adj vasculopathy	US-PGPUB; USPAT	OR	ON	2016/09/21 09:12
L5	0	polyploidal adj choridal adj vasculopathy	US-PGPUB; USPAT	OR	ON	2016/09/21 09:12
L6	1	"9062105".pn.	US-PGPUB; USPAT	OR	ON	2016/09/21 09:24
L7	1	"9062105".pn. and age$1related	US-PGPUB; USPAT	OR	ON	2016/09/21 09:24
L8	1	"20160144025" and cdr	US-PGPUB; USPAT	OR	ON	2016/09/21 09:49
L9	1	"20160144025" and cdr same ang	US-PGPUB; USPAT	OR	ON	2016/09/21 09:49
L10	1	"20120189635".pn.	US-PGPUB; USPAT	OR	ON	2016/09/21 10:09
L11	1	"20120189635".pn. and "18" and "20"	US-PGPUB; USPAT	OR	ON	2016/09/21 10:10
L12	1	"9062105".pn. and aflibercept	US-PGPUB; USPAT	OR	ON	2016/09/21 10:40
S1	1	"20160144025"	US-PGPUB; USPAT	OR	ON	2016/09/20 09:34
S2	0	"20160144025" and anti$1ang$1"2"$1inhibitor	US-PGPUB; USPAT	OR	ON	2016/09/20 09:35
S3	1	"20160144025" and ang adj2 inhibitor	US-PGPUB; USPAT	OR	ON	2016/09/20 09:36
S4	1	"20160144025" and (ang or angiopoietin) adj2 (inhibitor or antibod$3)	US-PGPUB;	OR	ON	2016/09/20 09:37

			USPAT			
S5	12	("7531173" "7303748" "7521049" "7306799" "7303747" "8921316" "8110546" "8802107" "9345904" "8481046" "8092803" "7608261").pn.	US-PGPUB; USPAT	OR	ON	2016/09/20 11:08
S6	0	("7531173" "7303748" "7521049" "7306799" "7303747" "8921316" "8110546" "8802107" "9345904" "8481046" "8092803" "7608261").pn. and (ang same vegf)	US-PGPUB; USPAT	OR	ON	2016/09/20 11:16
S7	0	("7531173" "7303748" "7521049" "7306799" "7303747" "8921316" "8110546" "8802107" "9345904" "8481046" "8092803" "7608261").pn. and (angiopoietin and vegf)	US-PGPUB; USPAT	OR	ON	2016/09/20 11:18
S8	0	("7531173" "7303748" "7521049" "7306799" "7303747" "8921316" "8110546" "8802107" "9345904" "8481046" "8092803" "7608261").pn. and (angiopoietin same intraocular)	US-PGPUB; USPAT	OR	ON	2016/09/20 11:18
S9	0	("7531173" "7303748" "7521049" "7306799" "7303747" "8921316" "8110546" "8802107" "9345904" "8481046" "8092803" "7608261").pn. and ((angiopoietin or ang$2) same intraocular)	US-PGPUB; USPAT	OR	ON	2016/09/20 11:18
S10	3	("7531173" "7303748" "7521049" "7306799" "7303747" "8921316" "8110546" "8802107" "9345904" "8481046" "8092803" "7608261").pn. and ((vegf) same intraocular)	US-PGPUB; USPAT	OR	ON	2016/09/20 11:18
S11	1167	aflibercept	US-PGPUB; USPAT	OR	ON	2016/09/20 11:41
S12	363	aflibercept and (eye or ocular or retina or retinopathy or macular).clm.	US-PGPUB; USPAT	OR	ON	2016/09/20 11:42
S13	147	aflibercept and (eye or ocular or retina or retinopathy or macular).clm. and vegf.clm.	US-PGPUB; USPAT	OR	ON	2016/09/20 11:42
S14	60	aflibercept and (eye or ocular or retina or retinopathy or macular).clm. and vegf.clm. and treatment.clm.	US-PGPUB; USPAT	OR	ON	2016/09/20 11:42
S15	11	aflibercept and (eye or ocular or retina or retinopathy or macular).clm. and vegf.clm. and treatment.clm.	USPAT	OR	ON	2016/09/20 11:43
S16	1	"9320647".pn.	USPAT	OR	ON	2016/09/20 11:51
S17	1	"9320647".pn. and (eye or ocular or retina or retinopathy or macular)	USPAT	OR	ON	2016/09/20 11:52
S18	20	(ang2 or angiotensin) and (eye or ocular or retina or retinopathy or macular).clm. and vegf.clm. and treatment.clm.	USPAT	OR	ON	2016/09/20 11:58
S19	4	(ang2 or angiotensin).clm. and (eye or ocular or retina or retinopathy or macular).clm. and vegf.clm. and treatment.clm.	USPAT	OR	ON	2016/09/20 11:58
S20	84	(ang2 or angiotensin).clm. and (eye or ocular or retina or retinopathy or macular).clm.	USPAT	OR	ON	2016/09/20 12:00
S21	32	(ang2 or angiotensin).clm. and (eye or ocular or retina or retinopathy or macular).clm. and treatment.clm.	USPAT	OR	ON	2016/09/20 12:00

S22	1	(ang2 or angiotensin) and "9062105".pn.	USPAT	OR	ON	2016/09/20 13:01
S23	0	"9320647".pn. and dosage	USPAT	OR	ON	2016/09/20 13:07
S24	1	"9320647".pn. and amount	USPAT	OR	ON	2016/09/20 13:07
S25	0	("7531173" "7303748" "7521049" "7306799" "7303747" "8921316" "8110546" "8802107" "9345904" "8481046" "8092803" "7608261").pn. and angiotensin	US-PGPUB; USPAT	OR	ON	2016/09/20 13:19
S26	1	"8747852".pn. and aflibercept	US-PGPUB; USPAT	OR	ON	2016/09/20 13:23
S27	0	"8747852".pn. and angiotensin	US-PGPUB; USPAT	OR	ON	2016/09/20 13:27
S28	0	"8747852".pn. and angiotensen	US-PGPUB; USPAT	OR	ON	2016/09/20 13:27
S29	0	"8657804".pn. and (ang or angiopoietin)	US-PGPUB; USPAT	OR	ON	2016/09/20 13:29
S30	0	"8747852".pn. and angiopoietin	US-PGPUB; USPAT	OR	ON	2016/09/20 13:29
S31	0	"8521273".pn. and angiopoietin	US-PGPUB; USPAT	OR	ON	2016/09/20 13:30
S32	20	(ang2 or angiotensin) and (eye or ocular or retina or retinopathy or macular).clm. and vegf.clm. and treatment.clm. not S5	USPAT	OR	ON	2016/09/20 13:30

9/21/2016 10:48:40 AM
C:\Users\Ispector\Documents\EAST\Workspaces\14943490.wsp

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14943490
	Filing Date	2015-11-17
	First Named Inventor	Vitti, Robert L.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-020

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20130129722		2013-05-23	Lowy et al.	
	2	20140017244		2014-01-16	Duerr et al.	

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	Application Number	14943490
	Filing Date	2015-11-17
	First Named Inventor	Vitti, Robert L.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-020

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

Examiner Signature	/LORRAINE SPECTOR/	Date Considered	09/20/2016

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14943490
	Filing Date	2015-11-17
	First Named Inventor	Vitti, Robert L.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-020

CERTIFICATION STATEMENT

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

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

OR

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

X See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Karl Bozicevic, Reg. No. 28,807/	Date (YYYY-MM-DD)	2016-04-20
Name/Print	Karl Bozicevic	Registration Number	28807

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

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

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

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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.

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.

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United States Patent and Trademark Office
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/943,490	11/17/2015	Robert L. VITTI	REGN-020

CONFIRMATION NO. 1006

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

PUBLICATION NOTICE

OC000000083209198

Title:METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES

Publication No.US-2016-0144025-A1
Publication Date:05/26/2016

NOTICE OF PUBLICATION OF APPLICATION

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

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

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

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

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

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/943,490	11/17/2015	Robert L. VITTI	REGN-020

CONFIRMATION NO. 1006

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

POA ACCEPTANCE LETTER

OC000000083138186

Date Mailed: 05/25/2016

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/13/2016.

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**.

/sibrahim/

Doc Code: PA..
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
14/943,490	November 17, 2015

(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:

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Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

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

Regeneron Pharmaceuticals, Inc.

☐ Inventor or Joint Inventor (title not required below)

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

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

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

SIGNATURE of Applicant for Patent

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

Signature	/Frank R. Cottingham/	Date (Optional)	
Name	Frank R. Cottingham		
Title	Senior Director, Assistant General Counsel, Patents		

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.**

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Electronic Acknowledgement Receipt	
EFS ID:	25766232
Application Number:	14943490
International Application Number:	
Confirmation Number:	1006
Title of Invention:	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES
First Named Inventor/Applicant Name:	Robert L. VITTI
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-020
Receipt Date:	13-MAY-2016
Filing Date:	17-NOV-2015
Time Stamp:	16:40:29
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Assignee showing of ownership per 37 CFR 3.73	REGN-020_373_c.pdf	32208 a34ad7f9325dacf8ca18664f5bdc782c48b5c29d	no	2

Warnings:
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2	Power of Attorney	REGN-020_POA.pdf	91630 75c7ffeb70b6f20aa361aea589d5eab2dcf4cb13	no	1

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Total Files Size (in bytes):	123838

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

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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
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STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: **Robert L. Vitti et al.**

Application No./Patent No.: **14/943,490** Filed/Issue Date: **November 17, 2015**

Titled: **Methods and Formulations for Treating Vascular Eye Diseases**

REGENERON PHARMACEUTICALS, INC., a **corporation**
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

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

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

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

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

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

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

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

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

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

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

A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel **038438**, Frame **0854**, 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: _____

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STATEMENT UNDER 37 CFR 3.73(c)

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☐ 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/	**May 13, 2016**
Signature	Date
Karl Bozicevic	**28,807**
Printed or Typed Name	Title or Registration Number

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14943490
	Filing Date	2015-11-17
	First Named Inventor	Vitti, Robert L.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-020

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	1	20130129722		2013-05-23	Lowy et al.	
	2	20140017244		2014-01-16	Duerr et al.	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14943490
	Filing Date	2015-11-17
	First Named Inventor	Vitti, Robert L.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-020

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

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14943490
	Filing Date	2015-11-17
	First Named Inventor	Vitti, Robert L.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-020

CERTIFICATION STATEMENT

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

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

OR

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

☒ See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Karl Bozicevic, Reg. No. 28,807/	Date (YYYY-MM-DD)	2016-04-20
Name/Print	Karl Bozicevic	Registration Number	28807

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

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

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

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

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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.

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.

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EFS ID:	25536961
Application Number:	14943490
International Application Number:	
Confirmation Number:	1006
Title of Invention:	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES
First Named Inventor/Applicant Name:	Robert L. VITTI
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-020
Receipt Date:	20-APR-2016
Filing Date:	17-NOV-2015
Time Stamp:	13:00:58
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-020_SUPP_IDS_transmittal_04-20-2016.pdf	52767 5d44c360bb507e6674567b2413567e3ba1484bf3	no	2

Warnings:
Information:

2	Information Disclosure Statement (IDS) Form (SB08)	REGN-020_Supp_IDS_SB08.pdf	612213 9483001bd19577bea33a6290e53bbb7b13138e6c	no	4
Warnings:					
Information:					
			Total Files Size (in bytes):	664980	

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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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INFORMATION DISCLOSURE STATEMENT Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450		
	Attorney Docket	REGN-020
	First Named Inventor	Vitti, Robert L.
	Application Number	14/943,490
	Confirmation No.	1006
	Filing Date	November 17, 2015
	Group Art Unit	
	Examiner Name	
	Title: "*Methods and Formulations for Treating Vascular Eye Diseases*"	

Sir:

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

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

Statements

☐ **No statement**

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

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

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

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

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

Fees

☒ No fee is believed to be due.

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

The Commissioner is hereby authorized to charge any underpayment of fees up to a strict limit of $3,000.00 beyond that authorized on the credit card, but not more than $3,000.00 in additional fees due with any communication for the above referenced patent application, including but not limited to any necessary fees for extensions of time, or credit any overpayment of any amount to Deposit Account No. 50-0815, order number REGN-020.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: April 20, 2016

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

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, California 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	Methods and Formulations for Treating Vascular Eye Diseases

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 14/943,490

filed on November 17, 2015

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 five (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: Robert L. VITTI Date (Optional): 4 Jan 2016

Signature: Robert L. Vitti

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. 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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filed on November 17, 2015.

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

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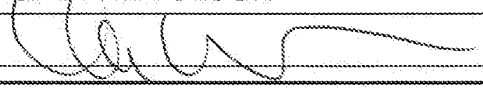
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LEGAL NAME OF INVENTOR

Inventor: Kristine A. ERICKSON Date (Optional): 7-Jan-2016

Signature: 

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. 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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This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 14/943,490

filed on November 17, 2015.

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.

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LEGAL NAME OF INVENTOR

Inventor: Karen W. CHU Date (Optional): _____

Signature: _____

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

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LEGAL NAME OF INVENTOR

Inventor: Stanley J. WIEGAND Date (Optional): 1/12/2016

Signature: Stanley J. Wiegand

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

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LEGAL NAME OF INVENTOR

Inventor: Jingtai CAO Date (Optional): _____

Signature: _____

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. 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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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	Methods and Formulations for Treating Vascular Eye Diseases

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 14/943,490

filed on November 17, 2015.

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 five (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: Ivan B. LOBOV Date (Optional): 01/25/2016

Signature: [signature]

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

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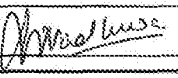
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LEGAL NAME OF INVENTOR

Inventor: Saurabh WADHWA Date (Optional): 04 Jan 2016

Signature: 

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LEGAL NAME OF INVENTOR

Inventor: Kenneth S. GRAHAM Date (Optional): 30 March 2015

Signature: Kenneth S Graham

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

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LEGAL NAME OF INVENTOR

Inventor: Daniel DIX Date (Optional): Jan 04, 2016

Signature: Daniel P. Dix

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

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
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EFS ID:	25500969
Application Number:	14943490
International Application Number:	
Confirmation Number:	1006
Title of Invention:	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES
First Named Inventor/Applicant Name:	Robert L. VITTI
Customer Number:	96387
Filer:	Aparna Patankar
Filer Authorized By:	
Attorney Docket Number:	REGN-020
Receipt Date:	18-APR-2016
Filing Date:	17-NOV-2015
Time Stamp:	11:37:41
Application Type:	Utility under 35 USC 111(a)

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	Oath or Declaration filed	10112US01_FinalDeclaration.pdf	4937745 e8b241442d0b3f7e0ab695678f8b9b643543700b	no	10
Warnings:					
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Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14943490
	Filing Date	2015-11-17
	First Named Inventor	Vitti, Robert L.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-020

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	Attorney Docket Number	REGN-020

	1	Koenig et al. "Deep Sequencing-guided Design of a High Affinity Dual Specificity Antibody to Target Two Angiogenic Factors in Neovascular Age-related Macular Degeneration" Journal of Biological Chemistry, September 4, 2015, Vol. 290, No. 36. pp. 21773-21786.	
	2	Oliner et al. "AMG 386, a Selective Angiopoietin 1/2-Neutralizing Peptibody, Inhibits Angiogenesis in Models of Ocular Neovascular Diseases" Investigative Ophthalmology & Visual Science, April 2012, Vol. 53, No. 4, pp. 2170-2180.	
	3	Rennel et al. "A Human Neutralizing Antibody Specific to Ang-2 Inhibits Ocular Angiogenesis" 2011, Microcirculation, Vol. 18, pp. 598-607.	
	4	Rubio et al. "Ocular Angiogenesis: Vascular Endothelial Growth Factor and Other Factors" Retinal Pharmacotherapeutics, 2016, Vol. 55, pp. 28-37.	
	5	Tuuminen et al. "Increased Intravitreal angiopoietin-2 levels in patients with retinal vein occlusion" 2014, pp. 164-165.	
	6	You et al. "Effects of laser photocoagulation on serum agiopoietin-1, agiopoietin-2, angiopoietin-1/angiopoietin-2 ratio,, and soluble angiopoietin receptor Tie-2 levels in type 2 diabetic patients with proliferative diabetic retinopathy." International Journal of Ophthalmology, Vol. 7, No. 4, August 18, 2014, pp. 648-653.	

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Examiner Signature		Date Considered	

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

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

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	First Named Inventor	Vitti, Robert L.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	REGN-020

CERTIFICATION STATEMENT

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

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

OR

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

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Karl Bozicevic, Reg. No. 28,807/	Date (YYYY-MM-DD)	2016-03-02
Name/Print	Karl Bozicevic	Registration Number	28807

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(73) Proprietor: **F. Hoffmann-La Roche AG**
4070 Basel (CH)

(72) Inventors:
- **BAEHNER, Monika**
  81547 München (DE)
- **BRINKMANN, Ulrich**
  82362 Weilheim (DE)
- **GEORGES, Guy**
  82392 Habach (DE)
- **GRIEP, Remko Albert**
  3470 Slemmestad (NO)
- **IMHOF-JUNG, Sabine**
  82152 Planegg (DE)
- **KAVLIE, Anita**
  0287 Oslo (NO)
- **KETTENBERGER, Hubert**
  81369 Muenchen (DE)
- **KLEIN, Christian**
  8906 Bonstetten (CH)
- **REGULA, Joerg, Thomas**
  80639 München (DE)
- **SCHAEFER, Wolfgang**
  68199 Mannheim (DE)
- **SCHANZER, Juergen Michael**
  81667 München (DE)
- **SCHEUER, Werner**
  82377 Penzberg (DE)
- **SEEBER, Stefan**
  82404 Sindelsdorf (DE)
- **THOMAS, Markus**
  82377 Penzberg (DE)

(74) Representative: **Burger, Alexander et al**
Roche Diagnostics GmbH
Patent Department (TR-E)
Postfach 11 52
82372 Penzberg (DE)

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Description

[0001] The present invention relates to bispecific antibodies against human vascular endothelial growth factor (VEGF/VEGF-A) and against human angiopoietin-2 (ANG-2), methods for their production, pharmaceutical compositions containing said antibodies, and uses thereof.

Background of the Invention

[0002] Angiogenesis is implicated in the pathogenesis of a variety of disorders which include solid tumors, intraocular neovascular syndromes such as proliferative retinopathies or age-related macular degeneration (AMD), rheumatoid arthritis, and psoriasis (Folkman, J., et al., J. Biol. Chem. 267 (1992) 10931-10934; Klagsbrun, M., et al., Annu. Rev. Physiol. 53 (1991) 217-239; and Garner, A., Vascular diseases, in: Pathobiology of ocular disease, A dynamic approach, Garner, A., and Klintworth, G. K. (eds.), 2nd edition, Marcel Dekker, New York (1994), pp 1625-1710). In the case of solid tumors, the neovascularization allows the tumor cells to acquire a growth advantage and proliferative autonomy compared to the normal cells. Accordingly, a correlation has been observed between density of microvessels in tumor sections and patient survival in breast cancer as well as in several other tumors (Weidner, N., et al., N Engl J Med. 324 (1991) 1-8; Horak, E.R., et al., Lancet 340 (1992) 1120-1124; and Macchiarini, P., et al., Lancet 340 (1992) 145-146).

VEGF and anti-VEGF antibodies

[0003] Human vascular endothelial growth factor (VEGF/VEGF-A) (SEQ ID No: 105) is described in e.g. Leung, D.W., et al., Science 246 (1989) 1306-9; Keck, P.J., et al., Science 246 (1989) 1309-12 and Connolly, D.T., et al., J. Biol. Chem. 264 (1989) 20017-24. VEGF is involved in the regulation of normal and abnormal angiogenesis and neovascularization associated with tumors and intraocular disorders (Ferrara, N., et al., Endocr. Rev. 18 (1997) 4-25; Berkman, R.A.,et al., J. Clin. Invest. 91 (1993) 153-159; Brown, L.F., et al., Human Pathol. 26 (1995) 86-91; Brown, L.F., et al., Cancer Res. 53 (1993) 4727-4735; Mattern, J., et al., Brit. J. Cancer. 73 (1996) 931-934; and Dvorak, H., et al., Am. J. Pathol. 146 (1995) 1029-1039). VEGF is a homodimeric glycoprotein that has been isolated from several sources. VEGF shows highly specific mitogenic activity for endothelial cells. VEGF has important regulatory functions in the formation of new blood vessels during embryonic vasculogenesis and in angiogenesis during adult life (Carmeliet, P., et al., Nature, 380 (1996) 435-439; Ferrara, N., et al., Nature, 380 (1996) 439-442; reviewed in Ferrara and Davis-Smyth, Endocrine Rev., 18 (1997) 4-25. The significance of the role played by VEGF has been demonstrated in studies showing that inactivation of a single VEGF allele results in embryonic lethality due to failed development of the vasculature (Carmeliet, P., et al., Nature, 380 (1996) 435-439; Ferrara, N., et al., Nature, 380 (1996) 439-442. In addition VEGF has strong chemoattractant activity towards monocytes, can induce the plasminogen activator and the plasminogen activator inhibitor in endothelial cells, and can also induce microvascular permeability. Because of the latter activity, it is sometimes referred to as vascular permeability factor (VPF). The isolation and properties of VEGF have been reviewed; see Ferrara, N., et al., J. Cellular Biochem., 47 (1991) 211-218 and Connolly, J. Cellular Biochem., 47 (1991) 219-223. Alternative mRNA splicing of a single VEGF gene gives rise to five isoforms of VEGF.

[0004] Anti-VEGF neutralizing antibodies suppress the growth of a variety of human tumor cell lines in mice (Kim, I., et al., Nature 362 (1993) 841-844; Warren, S.R., et al., J. Clin. Invest. 95 (1995) 1789-1797; Borgstrom, P., et al., Cancer Res. 56 (1996) 4032-4039; and Melnyk, O., et al., Cancer Res. 56 (1996) 921-924). WO 94/10202, WO 98/45332, WO 2005/00900 and WO 00/35956 refer to antibodies against VEGF. Humanized monoclonal antibody bevacizumab (sold under the trade name Avastin®) is an anti-VEGF antibody used in tumor therapy WO 98/45331).

[0005] Ranibizumab (trade name Lucentis®) is a monoclonal antibody fragment derived from the same parent murine antibody as bevacizumab (Avastin). It is much smaller than the parent molecule and has been affinity matured to provide stronger binding to VEGF-A (WO 98/45331). It is an anti-angiogenic that has been approved to treat the "wet" type of age-related macular degeneration (ARMD), a common form of age-related vision loss. Another anti-VEGF antibody is e.g. HuMab G6-31 described e.g. in US 2007/0141065.

ANG-2 and anti-ANG-2 antibodies

[0006] Human angiopoietin-2 (ANG-2) (alternatively abbreviated with ANGPT2 or ANG2) (SEQ ID No: 106) is described in Maisonpierre, P.C., et al, Science 277 (1997) 55-60 and Cheung,A.H., et al., Genomics 48 (1998) 389-91. The angiopoietins-1 and -2 (ANG-1 (SEQ ID No: 107) and ANG-2 (SEQ ID No: 106)) were discovered as ligands for the Ties, a family of tyrosine kinases that is selectively expressed within the vascular endothelium. Yancopoulos, G.D., et al., Nature 407 (2000) 242-48. There are now four definitive members of the angiopoietin family. Angiopoietin-3 and -4 (Ang-3 and Ang-4) may represent widely diverged counterparts of the same gene locus in mouse and man. Kim, I., et al., FEBS Let, 443 (1999) 353-56; Kim, I., et al., J Biol Chem 274 (1999) 26523-28. ANG-1 and ANG-2 were originally

identified in tissue culture experiments as agonist and antagonist, respectively (see for ANG-1: Davis, S., et al., Cell 87 (1996) 1161-69; and for ANG-2: Maisonpierre, P.C., et al., Science 277 (1997) 55-60) All of the known angiopoietins bind primarily to Tie2, and both Ang-1 and -2 bind to Tie2 with an affinity of 3 nM (Kd). Maisonpierre, P.C., et al., Science 277 (1997) 55-60. Ang-1 was shown to support EC survival and to promote endothelium integrity, Davis, S., et al., Cell 87 (1996) 1161-69; Kwak, H.J., et al., FEBS Lett 448 (1999) 249-53; Suri, C., et al., Science 282 (1998) 468-71; Thurston, G., et al., Science 286 (1999) 251 1-14; Thurston, G., et al., Nat. Med. 6 (2000) 460-63, whereas ANG-2 had the opposite effect and promoted blood vessel destabilization and regression in the absence of the survival factors VEGF or basic fibroblast growth factor. Maisonpierre, P.C., et al., Science 277 (1997) 55-60. However, many studies of ANG-2 function have suggested a more complex situation. ANG-2 might be a complex regulator of vascular remodeling that plays a role in both vessel sprouting and vessel regression. Supporting such roles for ANG-2, expression analyses reveal that ANG-2 is rapidly induced, together with VEGF, in adult settings of angiogenic sprouting, whereas ANG-2 is induced in the absence of VEGF in settings of vascular regression. Holash, J., et al., Science 284 (1999) 1994-98; Holash, J., et al., Oncogene 18 (1999) 5356-62. Consistent with a context-dependent role, ANG-2 specifically binds to the same endothelial-specific receptor, Tie-2, which is activated by Ang-1, but has context-dependent effects on its activation. Maisonpierre, P.C., et al., Science 277 (1997) 55-60.

[0007] Corneal angiogenesis assays have shown that both ANG-1 and ANG-2 had similar effects, acting synergistically with VEGF to promote growth of new blood vessels. Asahara, T., et al., Circ. Res. 83 (1998) 233-40. The possibility that there was a dose-dependent endothelial response was raised by the observation that in vitro at high concentration, ANG-2 can also be pro-angiogenic. Kim, I., et al., Oncogene 19 (2000) 4549-52. At high concentration, ANG-2 acts as an apoptosis survival factor for endothelial cells during serum deprivation apoptosis through activation of Tie2 via PI-3 Kinase and Akt pathway. Kim, I., et al., Oncogene 19 (2000) 4549-52.

[0008] Other in vitro experiments suggested that during sustained exposure, the effects of ANG-2 may progressively shift from that of an antagonist to an agonist of Tie2, and at later time points, it may contribute directly to vascular tube formation and neovessel stabilization. Teichert-Kuliszewska, K., et al., Cardiovasc. Res. 49 (2001) 659-70. Furthermore, if ECs were cultivated on fibrin gel, activation of Tie2 with ANG-2 was also observed, perhaps suggesting that the action of ANG-2 could depend on EC differentiation state. Teichert-Kuliszewska, K., et al., Cardiovasc. Res. 49 (2001) 659-70. In microvascular EC cultured in a three-dimensional collagen gel, ANG-2 can also induce Tie2 activation and promote formation of capillary-like structures. Mochizuki, Y., et al., J. Cell. Sci. 115 (2002) 175-83. Use of a 3-D spheroidal coculture as an in-vitro model of vessel maturation demonstrated that direct contact between ECs and mesenchymal cells abrogates responsiveness to VEGF, whereas the presence of VEGF and ANG-2 induced sprouting. Korff, T., et al., Faseb J. 15 (2001) 447-57 . Etoh, T.H. et al. demonstrated that ECs that constitutively express Tie2, the expression of MMP-1, -9 and u-PA were strongly upregulated by ANG-2 in the presence of VEGF. Etoh, T., et al., Cancer Res. 61 (2001) 2145-53. With an in vivo pupillary membrane model, Lobov, I.B. et al. showed that ANG-2 in the presence of endogenous VEGF promotes a rapid increase in capillary diameter, remodeling of the basal lamina, proliferation and migration of endothelial cells, and stimulates sprouting of new blood vessels. Lobov, I.B., et al., Proc. Natl. Acad. Sci. USA 99 (2002) 11205-10. By contrast, ANG-2 promotes endothelial cell death and vessel regression without endogenous VEGF. Lobov, I.B., et al., Proc. Natl. Acad. Sci. USA 99 (2002) 11205-10. Similarly, with an in vivo tumor model, Vajkoczy, P., et al. demonstrated that multicellular aggregates initiate vascular growth by angiogenic sprouting via the simultaneous expression of VEGFR-2 and ANG-2 by host and tumor endothelium. Vajkoczy, P., et al., J. Clin. Invest. 109 (2002) 777-85. This model illustrated that the established microvasculature of growing tumors is characterized by a continuous remodeling, putatively mediated by the expression of VEGF and ANG-2. Vajkoczy, P., et al., J Clin. Invest. 09 (2002) 777-85.

[0009] Knock-out mouse studies of Tie-2 and Angiopoietin-1 show similar phenotypes and suggest that Angiopoietin-1 stimulated Tie-2 phosphorylation mediates remodeling and stabilization of developing vessel, promoting blood vessel maturation during angiogenesis and maintenance of endothelial cell-support cell adhesion (Dumont, J., et al., Genes & Development, 8 (1994) 1897-1909; Sato, T.N., Nature, 376 (1995) 70-74; (Thurston, G., et al., Nature Medicine: 6 (2000) 460-463). The role of Angiopoietin-1 is thought to be conserved in the adult, where it is expressed widely and constitutively (Hanahan, D., Science, 277 (1997) 48-50; Zagzag, D., et al., Exp Neurology, 159:391-400 (1999)). In contrast, Angiopoietin-2 expression is primarily limited to sites of vascular remodeling where it is thought to block the constitutive stabilizing or maturing function of Angiopoietin-1, allowing vessels to revert to, and remain in, a plastic state which may be more responsive to sprouting signals (Hanahan, D., 1997; Holash, J., et al., Orzcogerze 18 (199) 5356-62; Maisonpierre, P.C., 1997). Studies of Angiopoietin-2 expression in pathological angiogenesis have found many tumor types to show vascular Angiopoetin-2 expression (Maisonpierre, P.C., et al., Science 277 (1997) 55-60). Functional studies suggest Angiopoietin-2 is involved in tumor angiogenesis and associate Angiopoietin-2 overexpression with increased tumor growth in a mouse xenograft model (Ahmad, S.A., et al., Cancer Res., 61 (2001) 1255-1259). Other studies have associated Angiopoietin-2 overexpression with tumor hypervascularity (Etoh, T., et al., Cancer Res. 61 (2001) 2145-53; Tanaka, F., et al., Cancer Res. 62 (2002) 124-29).

[0010] In recent years Angiopoietin-1, Angiopoietin-2 and/or Tie-2 have been proposed as possible anti-cancer ther-

apeutic targets. For example US 6,166,185, US 5,650,490 and US 5,814,464 each disclose anti-Tie-2 ligand and receptor antibodies. Studies using soluble Tie-2 were reported to decrease the number and size of tumors in rodents (Lin, 1997; Lin 1998). Siemester, G., et al. Siemeister, G., et al., Cancer Res. 59 (1999) 3185-91 generated human melanoma cell lines expressing the extracellular domain of Tie-2, injected these into nude mice and reported soluble Tie-2 to result in significant inhibition of tumor growth and tumor angiogenesis. Given both Angiopoietin-1 and Angiopoietin-2 bind to Tie-2, it is unclear from these studies whether Angiopoietin-1, Angiopoietin-2 or Tie-2 would be an attractive target for anti-cancer therapy. However, effective anti-Angiopoietin-2 therapy is thought to be of benefit in treating diseases such as cancer, in which progression is dependant on aberrant angiogenesis where blocking the process can lead to prevention of disease advancement (Folkunan, J., Nature Medicine. 1 (1995) 27-31).

[0011] In addition some groups have reported the use of antibodies and peptides that bind to Angiopoietin-2. See, for example, US 6 166,185 and US 2003/10124129. WO 03/030833, WO 2006/068953, WO 03/057134 or US 2006/0122370.

[0012] Study of the effect of focal expression of Angiopoietin-2 has shown that antagonizing the Angiopoietin-1/Tie-2 signal loosens the tight vascular structure thereby exposing ECs to activating signals from angiogenesis inducers, e.g. VEGF (Hanahan, D., Science, 277 (1997) 48-50). This pro-angiogenic effect resulting from inhibition of Angiopoietin-1 indicates that anti-Angiopoietin-1 therapy would not be an effective anti-cancer treatment.

[0013] ANG-2 is expressed during development at sites where blood vessel remodeling is occurring. Maisonpierre, P.C., et al., Science 277 (1997) 55-60. In adult individuals, ANG-2 expression is restricted to sites of vascular remodeling as well as in highly vascularized tumors, including glioma, Osada, H., et al., Int. J. Oncol. 18 (2001) 305-09); Koga, K., et al., Cancer Res. 61 (2001) 6248-54, hepatocellular carcinoma, Tanaka, S., et al., J. Clin. Invest. 103 (1999) 341-45, gastric carcinoma, Etoh, T., et al., Cancer Res. 61 (2001) 2145-53; Lee, J.H., et al.,, Int. J. Oncol. 18 (2001) 355-61, thyroid tumor, Bunone, G., et al., Am J Pathol 155 (1999) 1967-76 non-small cell lung cancer, Wong, M.P., et al., Lung Cancer 29 (2000) 11-22, and cancer of colon, Ahmad, S.A., et al., Cancer 92 (2001) 1138-43, and prostate Wurmbach, J.H., et al., Anticancer Res. 20 (2000) 5217-20. Some tumor cells are found to express ANG-2. For example, Tanaka, S., et al., J. Clin. Invest. 103 (1999) 341-45 detected ANG-2 mRNA in 10 out of 12 specimens of human hepatocellular carcinoma (HCC). Ellis' group reported that ANG-2 is expressed ubiquitously in tumor epithelium. Ahmad, S.A., et al., Cancer 92 (2001) 1138-43. Other investigators reported similar findings. Chen, L., et al., J. Tongji Med. Univ. 21 (2001) 228-35. By detecting ANG-2 mRNA levels in archived human breast cancer specimens, Sfiligoi, C., et al., Int. J. Cancer 103 (2003) 466-74 reported that ANG-2 mRNA is significantly associated with auxiliary lymph node invasion, short disease-free time and poor overall survival. Tanaka, F., et al., Cancer Res. 62 (2002) 7124-29 reviewed a total of 236 patients of non-small cell lung cancer (NSCLC) with pathological stage-I to -IIIA, respectively. Using immunohistochemistry, they found that 16.9% of the NSCLC patients were ANG-2 positive. The microvessel density for ANG-2 positive tumor is significantly higher than that of ANG-2 negative. Such an angiogenic effect of ANG-2 was seen only when VEGF expression was high. Moreover, positive expression of ANG-2 was a significant factor to predict a poor postoperative survival. Tanaka, F., et al., Cancer Res. 62 (2002) 7124-29. However, they found no significant correlation between Ang-1 expression and the microvessel density. Tanaka, F., et al., Cancer Res. 62 (2002) 7124-29. These results suggest that ANG-2 is an indicator of poor prognosis patients with several types of cancer.

[0014] Recently, using an ANG-2 knockout mouse model, Yancopoulos' group reported that ANG-2 is required for postnatal angiogenesis. Gale, N.W., et al., Dev. Cell 3 (2002) 411-23. They showed that the developmentally programmed regression of the hyaloid vasculature in the eye does not occur in the ANG-2 knockout mice and their retinal blood vessels fail to sprout out from the central retinal artery. Gale, N.W., et al., Dev. Cell 3 (2002) 411-23. They also found that deletion of ANG-2 results in profound defects in the patterning and function of the lymphatic vasculature. Gale, N.W., et al., Dev. Cell 3 (200) 411-23. Genetic rescue with Ang-1 corrects the lymphatic, but not the angiogenesis defects. Gale, N.W., et al., Dev. Cell 3 (2002) 411-23.

[0015] Peters and his colleagues reported that soluble Tie2, when delivered either as recombinant protein or in a viral expression vector, inhibited in vivo growth of murine mammary carcinoma and melanoma in mouse models. Lin, P., et al., Proc. Natl. Acad. Sci. USA 95 (1998) 8829-34; Lin, P., et al., J. Clin. Invest. 100 (1997) 2072-78. Vascular densities in the tumor tissues so treated were greatly reduced. In addition, soluble Tie2 blocked angiogenesis in the rat corneal stimulated by tumor cell conditioned media. Lin, P., et al., J. Clin. Invest. 100 (1997) 2072-78. Furthermore, Isner and his team demonstrated that addition of ANG-2 to VEGF promoted significantly longer and more circumferential neovascularity than VEGF alone. Asahara, T., et al., Circ. Res. 83 (1998) 233-40. Excess soluble Tie2 receptor precluded modulation of VEGF-induced neovascularization by ANG-2. Asahara, T., et al., Circ. Res. 83 (1998) 233-40. Siemeister, G., et al., Cancer Res. 59 (1999) 3185-91 showed with nude mouse xenografts that overexpression of the extracellular ligand-binding domains of either Flt-1 or Tie2 in the xenografts results in significant inhibition of pathway could not be compensated by the other one, suggesting that the VEGF receptor pathway and the Tie2 pathway should be considered as two independent mediators essential for the process of in vivo angiogenesis. Siemeister, G., et al., Cancer Res. 59: 3 (1999) 3185-91. This is proven by a more recent publication by White, R., R., et al., , Proc. Natl. Acad. Sci. USA 100 (2003) 5028-33. In their study, it was demonstrated that a nuclease-resistant RNA aptamer that specifically binds and inhibits ANG-2 significantly inhibited neovascularization induced by bFGF in the rat corneal micropocket angiogenesis

model.

Bispecific antibodies

[0016] A wide variety of recombinant antibody formats have been developed in the recent past, e.g. tetravalent bispecific antibodies by fusion of, e.g., an IgG antibody format and single chain domains (see e.g. Coloma, M.J., et al., Nature Biotech 15 (1997) 159-163; WO 2001/077342; and Morrison, S.L., Nature Biotech 25 (2007) 1233-1234).

[0017] Also several other new formats wherein the antibody core structure (IgA, IgD, IgE, IgG or IgM) is no longer retained such as dia-, tria- or tetrabodies, minibodies, several single chain formats (scFv, Bis-scFv), which are capable of binding two or more antigens, have been developed (Holliger, P., et al., Nature Biotech 23 (2005) 1126-1136; Fischer, N., Léger, O., Pathobiology 74 (2007) 3-14; Shen, J., et al., Journal of Immunological Methods 318 (2007) 65-74; Wu, C., et al., Nature Biotech. 25 (2007) 1290-1297).

[0018] All such formats use linkers either to fuse the antibody core (IgA, IgD, IgE, IgG or IgM) to a further binding protein (e.g. scFv) or to fuse e.g. two Fab fragments or scFvs (Fischer, N., Léger, O., Pathobiology 74 (2007) 3-14). It has to be kept in mind that one may want to retain effector functions, such as e.g. complement-dependent cytotoxicity (CDC) or antibody dependent cellular cytotoxicity (ADCC), which are mediated through the Fc receptor binding, by maintaining a high degree of similarity to naturally occurring antibodies.

[0019] In WO 2007/024715 are reported dual variable domain immunoglobulins as engineered multivalent and multispecific binding proteins. A process for the preparation of biologically active antibody dimers is reported in US 6,897,044. Multivalent F_v antibody construct having at least four variable domains which are linked with each over via peptide linkers are reported in US 7,129,330. Dimeric and multimeric antigen binding structures are reported in US 2005/0079170. Tri- or tetra-valent monospecific antigen-binding protein comprising three or four Fab fragments bound to each other covalently by a connecting structure, which protein is not a natural immunoglobulin are reported in US 6,511,663. In WO 2006/020258 tetravalent bispecific antibodies are reported that can be efficiently expressed in prokaryotic and eukaryotic cells, and are useful in therapeutic and diagnostic methods. A method of separating or preferentially synthesizing dimers which are linked via at least one interchain disulfide linkage from dimers which are not linked via at least one interchain disulfide linkage from a mixture comprising the two types of polypeptide dimers is reported in US 2005/0163782. Bispecific tetravalent receptors are reported in US 5,959,083. Engineered antibodies with three or more functional antigen binding sites are reported in WO 2001/077342.

[0020] Multispecific and multivalent antigen-binding polypeptides are reported in WO 1997/001580. WO 1992/004053 reports homoconjugates, typically prepared from monoclonal antibodies of the IgG class which bind to the same antigenic determinant are covalently linked by synthetic cross-linking. Oligomeric monoclonal antibodies with high avidity for antigen are reported in WO 1991/06305 whereby the oligomers, typically of the IgG class, are secreted having two or more immunoglobulin monomers associated together to form tetravalent or hexavalent IgG molecules. Sheep-derived antibodies and engineered antibody constructs are reported in US 6,350,860, which can be used to treat diseases wherein interferon gamma activity is pathogenic. In US 2005/0100543 are reported targetable constructs that are multivalent carriers of bi-specific antibodies, i.e., each molecule of a targetable construct can serve as a carrier of two or more bi-specific antibodies. Genetically engineered bispecific tetravalent antibodies are reported in WO 1995/009917. In WO 2007/109254 stabilized binding molecules that consist of or comprise a stabilized scFv are reported.

Combination of VEGF and ANG-2 Inhibitors

[0021] WO 2007/068895 refers to a combination of an ANG-2 antagonist and a VEGF, KDR and/or FLTL antagonists. WO 2007/089445 refers to ANG-2 and VEGF inhibitor combinations.

[0022] WO 2003/106501 refers to fusion proteins binding to Angiopoetin and containing a multimerization domain. WO 2008/132568 fusion proteins binding to Angiopoetin and VEGF.

[0023] Jendreyko et al. (Klinische Pädiatrie, vol. 218 (2006) 143-151) disclose a bispecific single chain Fv antibody against VEGF-R2 and Tie-2, the receptor for angiopoietin-2.

Summary of the Invention

[0024] A first aspect of the current invention is a bispecific antibody as defined in the claims specifically binding to human vascular endothelial growth factor (VEGF) and human angiopoietin-2 (ANG-2) comprising a first antigen-binding site that specifically binds to human VEGF and a second antigen-binding site that specifically binds to human ANG-2.

[0025] The bispecific antibody according to the invention is characterized in that

ii) said first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO: 2, and a CDR1 region of SEQ ID NO:3, and in the light

chain variable domain a CDR3 region of SEQ ID NO: 4, a CDR2 region of SEQ ID NO:5, and a CDR1 region of SEQ ID NO:6; and

iii) said second antigen-binding site specifically binding to ANG-2 comprises in the heavy chain variable domain a CDR3 region of SEQ ID NO: 46, a CDR2 region of, SEQ ID NO: 47, and a CDR1 region of SEQ ID NO: 48, and in the light chain variable domain a CDR3 region of SEQ ID NO: 49, a CDR2 region of SEQ ID NO: 50, and a CDR1 region of SEQ ID NO: 51.

[0026] The bispecific antibody according to the invention is bivalent

[0027] A further aspect of the invention is a nucleic acid molecule encoding a chain of said bispecific antibody.

[0028] The invention further provides expression vectors containing said nucleic acid according to the invention capable of expressing said nucleic acid in a prokaryotic or eukaryotic host cell, and host cells containing such vectors for the recombinant production of an antibody according to the invention.

[0029] The invention further comprises a prokaryotic or eukaryotic host cell comprising a vector according to the invention.

[0030] The invention further comprises a method for the production of a bispecific antibody according to the invention, characterized by expressing a nucleic acid according to the invention in a prokaryotic or eukaryotic host cell and recovering said bispecific antibody from said cell or the cell culture supernatant. The invention further comprises the antibody obtained by such a recombinant method.

[0031] Still further aspects of the invention are a pharmaceutical composition comprising said bispecific antibody, said composition for the treatment of cancer, the use of said bispecific antibody for the manufacture of a medicament for the treatment of cancer, a method of treatment of patient suffering from cancer by administering said bispecific antibody to a patient in the need of such treatment.

[0032] The bispecific antibodies according to the invention show benefits for human patients in need of a VEGF and ANG-2 targeting therapy. The antibodies according to the invention have new and inventive properties causing a benefit for a patient suffering from such a disease, especially suffering from cancer. Surprisingly it has found out that the bispecific antibodies according to the invention are more effective in tumor growth and/or inhibition of tumor angiogenesis compared to combination of the respective monospecific parent antibodies.

Detailed Description of the Invention

[0033] The invention provides a bispecific antibody as defined in the claims specifically binding to human VEGF and human ANG-2 comprising a first antigen-binding site that specifically binds to human VEGF and a second antigen-binding site that specifically binds to human ANG-2, characterized in that

i) said antigen-binding sites are each a pair of an antibody heavy chain variable domain and an antibody light chain variable domain;

ii) said first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO: 2, and a CDR1 region of SEQ ID NO:3, and in the light chain variable domain a CDR3 region of SEQ ID NO: 4, a CDR2 region of SEQ ID NO:5, and a CDR1 region of SEQ ID NO:6; and

iii) said second antigen-binding site specifically binding to ANG-2 comprises in the heavy chain variable domain a CDR3 region of SEQ ID NO: 46, a CDR2 region of SEQ ID NO: 47, and a CDR1 region of SEQ ID NO: 48, and in the light chain variable domain a CDR3 region of SEQ ID NO: 49, a CDR2 region of SEQ ID NO: 50, and a CDR1 region of SEQ ID NO: 51.

[0034] The parent antibody, Ang2i_LC06, specifically binds to human ANG-2, but not to human ANG-1.

[0035] Bispecific antibodies specifically binding to human vascular endothelial growth factor (VEGF) and human angiopoietin-2 (ANG-2) but not to human ANG-1 can have improved properties such as e.g. biological or pharmacological activity, less toxicity, or pharmacokinetic profile, compared to bispecific antibodies specifically binding to human vascular endothelial growth factor (VEGF) and human angiopoietin-2 (ANG-2) as well as to human ANG-1.

[0036] Thus a preferred embodiment is a bispecific antibody specifically binding to human VEGF and human ANG-2 comprising a first antigen-binding site that specifically binds to human VEGF and a second antigen-binding site that specifically binds to human ANG-2, characterized in that the second antigen-binding site that is not specifically binding to human Angiopoetin 1 (ANG-1).

[0037] One embodiment of the invention is a bispecific antibody specifically binding to human vascular endothelial

growth factor (VEGF) and human angiopoietin-2 (ANG-2) comprising a first antigen-binding site that specifically binds to human VEGF and a second antigen-binding site that specifically binds to human ANG-2, characterized in that the ratio of the binding affinities KD (antigen-binding site specific for VEGF)/KD(antigen-binding site specific for ANG-2) is 1.0 - 10.0, preferably 1.5 -8.0 (In one embodiment 5.0-8.0) and preferably the absolute KD value is in the range of 10^{-8}-10^{-13} mol/l. KD values are determined in a ANG-2/VEGF binding BIACORE (see Example 2, and Fig 15A). As both proteins human VEGF and human ANG-2 are both present as soluble receptor ligands in human serum at approximately same concentrations, blocking of the said both receptor ligands by a bispecific characterized in that the ratio of the binding affinities KD (antigen-binding site specific for VEGF)/KD(antigen-binding site specific for ANG-2) is 1.0- 10.0, preferably 1.5 -8.0, and in one embodiment 5.0-8.0 can lead to improved properties with respect to the anti-angiogenic effects, tumor growth inhibition or resistance mechanism during the treatment of cancer or vascular diseases with such a bispecific antibody. Preferably said bispecific antibody characterized in that the ratio of the binding affinities KD (antigen-binding site specific for VEGF)/KD(antigen-binding site specific for ANG-2) is 1.0 - 10.0, preferably 1.5 -8.0 (In one embodiment 5.0-8.0) and and said said bispecific antibody comprises as first antigen-binding site specifically binding to VEGF a heavy chain variable domain of SEQ ID NO: 7, and a light chain variable domain of SEQ ID NO: 8, as said second antigen-binding site specifically binding to ANG-2 a) either a heavy chain variable domain of SEQ ID NO: 52, and a light chain variable domain of SEQ ID NO: 53 or b) a heavy chain variable domain of SEQ ID NO: 84, and a light chain variable domain of SEQ ID NO: 85.

[0038] As used herein, "antibody" refers to a binding protein that comprises antigen-binding sites. The terms "binding site" or "antigen-binding site" as used herein denotes the region(s) of an antibody molecule to which a ligand actually binds. The term "antigen-binding site" include antibody heavy chain variable domains (VH) and/or an antibody light chain variable domains (VL), or pairs of VH/VL, and can be derived from whole antibodies or antibody fragments such as single chain Fv, a VH domain and/or a VL domain, Fab, or (Fab)2. In one embodiment of the current invention each of the antigen-binding sites comprises an antibody heavy chain variable domain (VH) and/or an antibody light chain variable domain (VL), and preferably is formed by a pair consisting of an antibody light chain variable domain (VL) and an antibody heavy chain variable domain (VH).

[0039] The antigen-binding site, and especially heavy chain variable domains (VH) and/or antibody light chain variable domains (VL), that specifically bind to human vascular endothelial growth factor (VEGF) can be derived a) from known anti-VEGF antibodies such as Kim et al., Nature 362 (1993) 841-844; Warren, R.S., et al., J. Clin. Invest. 95 (1995) 1789-1797; Borgstrom, P., et al., Cancer Res. 56 (1996) 4032-4039; Melnyk, O., et al., Cancer Res. 56 (1996) 921-924). WO 94/10202, WO 98/45332, WO 2005/00900, WO 00/35956 and US 2007/0141065 or b) from new anti-VEGF antibodies obtained by de novo immunization methods using inter alia either the human VEGF protein or nucleic acid or fragments thereof or by phage display.

[0040] The antigen-binding site, and especially heavy chain variable domains (VH) and/or antibody light chain variable domains (VL), that specifically bind to human angiopoietin-2 (ANG-2) can be derived a) from known anti- ANG-2 antibodies such as WO 03/030833, WO 2006/068953, WO 2006/045049 or US 6,166,185; or b) from new anti-ANG-2 antibodies obtained e.g. by de novo immunization methods using inter alia either the human ANG-2 protein or nucleic acid or fragments thereof or by phage display.

[0041] Antibody specificity refers to selective recognition of the antibody for a particular epitope of an antigen. Natural antibodies, for example, are monospecific.

[0042] "Bispecific antibodies" according to the invention are antibodies which have two different antigen-binding specificities. Where an antibody has more than one specificity, the recognized epitopes may be associated with a single antigen or with more than one antigen. Antibodies of the present invention are specific for two different antigens, i.e. VEGF as first antigen and ANG-2 as second antigen.

[0043] The term "monospecific" antibody as used herein denotes an antibody that has one or more binding sites each of which bind to the same epitope of the same antigen. The term "valent" as used within the current application denotes the presence of a specified number of binding sites in an antibody molecule. As such, the terms "bivalent", "tetravalent", and "hexavalent" denote the presence of two binding site, four binding sites, and six binding sites, respectively, in an antibody molecule. The bispecific antibodies according to the invention are at least "bivalent" and may be "trivalent" or "multivalent" (e.g.("tetravalent" or "hexavalent"). The bispecific antibody according to the invention is bivalent.

[0044] Human vascular endothelial growth factor (VEGF/VEGF-A) (SEQ ID No: 105) is described in e.g. Leung, D.W., et al., Science 246 (1989) 1306-9; Keck, P.J., et al., Science 246 (1989) 1309-12 and Connolly, D.T., et al., J. Biol. Chem. 264 (1989) 20017-24. VEGF is involved in the regulation of normal and abnormal angiogenesis and neovascularization associated with tumors and intraocular disorders (Ferrara, N., et al., Endocr. Rev. 18 (1997) 4-25; Berkman, R.A.,et al., J. Clin. Invest. 91 (1993) 153-159; Brown, L.F., et al., Human Pathol. 26 (1995) 86-91; Brown, L.F., et al., Cancer Res. 53 (1993) 4727-4735; Mattern, J., et al., Brit. J. Cancer. 73 (1996) 931-934; and Dvorak, H., et al., Am. J. Pathol. 146 (1995) 1029-1039). VEGF is a homodimeric glycoprotein that has been isolated from several sources. VEGF shows highly specific mitogenic activity for endothelial cells.

[0045] Human angiopoietin-2 (ANG-2) (alternatively abbreviated with ANGPT2 or ANG2) (SEQ ID No: 106) is described

in Maisonpierre, P.C., et al, Science 277 (1997) 55-60 and Cheung, A.H., et al., Genomics 48 (1998) 389-91. The angiopoietins-1 and -2 (ANG-1(SEQ ID No: 107) and ANG-2(SEQ ID No: 106)) were discovered as ligands for the Ties, a family of tyrosine kinases that is selectively expressed within the vascular endothelium. Yancopoulos, G.D., et al., Nature 407 (2000) 242-48. There are now four definitive members of the angiopoietin family. Angiopoietin-3 and -4 (Ang-3 and Ang-4) may represent widely diverged counterparts of the same gene locus in mouse and man. Kim, I., et al., FEBS Let, 443 (1999) 353-56; Kim, I., et al., J Biol Chem 274 (1999) 26523-28. ANG-1 and ANG-2 were originally identified in tissue culture experiments as agonist and antagonist, respectively (see for ANG-1: Davis, S., et al., Cell 87 (1996) 1161-69; and for ANG-2: Maisonpierre, P.C., et al., Science 277 (1997) 55-60) All of the known angiopoietins bind primarily to Tie2, and both Ang-1 and -2 bind to Tie2 with an affinity of 3 nM (Kd). Maisonpierre, P.C., et al., Science 277 (1997) 55-60.

[0046] An antigen-binding site of an antibody of the invention contains six complementarity determining regions (CDRs) which contribute in varying degrees to the affinity of the binding site for antigen. There are three heavy chain variable domain CDRs (CDRH1, CDRH2 and CDRH3) and three light chain variable domain CDRs (CDRL1, CDRL2 and CDRL3). The extent of CDR and framework regions (FRs) is determined by comparison to a compiled database of amino acid sequences in which those regions have been defined according to variability among the sequences.

[0047] In certain embodiments, antibodies of the invention further comprise immunoglobulin constant regions of one or more immunoglobulin classes. Immunoglobulin classes include IgG, IgM, IgA, IgD, and IgE isotypes and, in the case of IgG and IgA, their subtypes.

[0048] The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of a single amino acid composition.

[0049] The term "chimeric antibody" refers to an antibody comprising a variable region, i.e., binding region, from one source or species and at least a portion of a constant region derived from a different source or species, usually prepared by recombinant DNA techniques. Chimeric antibodies comprising a murine variable region and a human constant region are preferred. Other preferred forms of "chimeric antibodies" encompassed by the present invention are those in which the constant region has been modified or changed from that of the original antibody to generate the properties according to the invention, especially in regard to Clq binding and/or Fc receptor (FcR) binding. Such chimeric antibodies are also referred to as "class-switched antibodies.". Chimeric antibodies are the product of expressed immunoglobulin genes comprising DNA segments encoding immunoglobulin variable regions and DNA segments encoding immunoglobulin constant regions. Methods for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques are well known in the art. See, e.g., Mornson, S.L., et al., Proc. Natl. Acad. Sci. USA 81 (1984) 6851-6855; US 5,202,238 and US 5,204,244.

[0050] The term "humanized antibody" refers to antibodies in which the framework or "complementarity determining regions" (CDR) have been modified to comprise the CDR of an immunoglobulin of different specificity as compared to that of the parent immunoglobulin. In a preferred embodiment, a murine CDR is grafted into the framework region of a human antibody to prepare the "humanized antibody." See, e.g., Riechmann, L., et al., Nature 332 (1988) 323-327; and Neuberger, M.S., et al., Nature 314 (1985) 268-270. Particularly preferred CDRs correspond to those representing sequences recognizing the antigens noted above for chimeric antibodies. Other forms of "humanized antibodies" encompassed by the present invention are those in which the constant region has been additionally modified or changed from that of the original antibody to generate the properties according to the invention, especially in regard to Clq binding and/or Fc receptor (FcR) binding.

[0051] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germ line immunoglobulin sequences. Human antibodies are well-known in the state of the art (van Dijk, M.A., and van de Winkel, J.G., Curr. Opin. Chem. Biol. 5 (2001) 368-374). Human antibodies can also be produced in transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire or a selection of human antibodies in the absence of endogenous immunoglobulin production. Transfer of the human germline immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, e.g., Jakobovits, A., et al., Proc. Natl. Acad. Sci. USA 90 (1993) 2551-2555; Jakobovits, A., et al., Nature 362 (1993) 255-258; Bruggemann, M., et al., Year Immunol. 7 (1993) 33-40). Human antibodies can also be produced in phage display libraries (Hoogenboom, H.R., and Winter, G., J. Mol. Biol. 227 (1992) 381-388; Marks, J.D., et al., J. Mol. Biol. 222 (1991) 581-597). The techniques of Cole, A., et al. and Boerner, P., et al. are also available for the preparation of human monoclonal antibodies (Cole, A., et al., Monoclonal Antibodies and Cancer Therapy, Liss, A.L., p. 77 (1985); and Boerner, P., et al., J. Immunol. 147 (1991) 86-95). As already mentioned for chimeric and humanized antibodies according to the invention the term "human antibody" as used herein also comprises such antibodies which are modified in the constant region to generate the properties according to the invention, especially in regard to Clq binding and/or FcR binding, e.g. by "class switching" i.e. change or mutation of Fc parts (e.g. from IgG 1 to IgG4 and/or IgG1/IgG4 mutation).

[0052] The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from a host cell such as

a NS0 or CHO cell or from an animal (e.g. a mouse) that is transgenic for human immunoglobulin genes or antibodies expressed using a recombinant expression vector transfected into a host cell. Such recombinant human antibodies have variable and constant regions in a rearranged form. The recombinant human antibodies according to the invention have been subjected to in vivo somatic hypermutation. Thus, the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germ line VH and VL sequences, may not naturally exist within the human antibody germ line repertoire in vivo.

[0053] The "variable domain" (variable domain of a light chain (VL), variable region of a heavy chain (VH) as used herein denotes each of the pair of light and heavy chains which is involved directly in binding the antibody to the antigen. The domains of variable human light and heavy chains have the same general structure and each domain comprises four framework (FR) regions whose sequences are widely conserved, connected by three "hypervariable regions" (or complementarity determining regions, CDRs). The framework regions adopt a β-sheet conformation and the CDRs may form loops connecting the β-sheet structure. The CDRs in each chain are held in their three-dimensional structure by the framework regions and form together with the CDRs from the other chain the antigen binding site. The antibody heavy and light chain CDR3 regions play a particularly important role in the binding specificity/affinity of the antibodies according to the invention and therefore provide a further object of the invention.

[0054] The terms "hypervariable region" or "antigen-binding portion of an antibody" when used herein refer to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from the "complementarity determining regions" or "CDRs". "Framework" or "FR" regions are those variable domain regions other than the hypervariable region residues as herein defined. Therefore, the light and heavy chains of an antibody comprise from N- to C-terminus the domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. CDRs on each chain are separated by such framework amino acids. Especially, CDR3 of the heavy chain is the region which contributes most to antigen binding. CDR and FR regions are determined according to the standard definition of Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991).

[0055] The bispecific antibodies according to the invention include, in addition, such antibodies having "conservative sequence modifications" (which is meant by "variants" of the bispecific antibodies). This means nucleotide and amino acid sequence modifications which do not affect or alter the above-mentioned characteristics of the antibody according to the invention. Modifications can be introduced by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g. lysine, arginine, histidine), acidic side chains (e.g. aspartic acid, glutamic acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g. alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g. threonine, valine, isoleucine) and aromatic side chains (e.g. tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a bispecific <VEGF-ANG-2> antibody can be preferably replaced with another amino acid residue from the same side chain family. A "variant" bispecific <VEGF-ANG-2> antibody, refers therefore herein to a molecule which differs in amino acid sequence from a "parent" bispecific <VEGF-ANG-2> antibody amino acid sequence by up to ten, preferably from about two to about five, additions, deletions and/or substitutions in one or more variable region or constant region of the parent antibody. Amino acid substitutions can be performed by mutagenesis based upon molecular modeling as described by Riechmann, L., et al., Nature 332 (1988) 323-327 and Queen, C., et al., Proc. Natl. Acad. Sci. USA 86 (1989) 10029-10033. A "variant" bispecific <VEGF-ANG-2> antibody according to the invention includes also bispecific antibodies formats in which the linker (if existing) was modified, or replaced by another linker.

[0056] As used herein, the term "binding" or "specifically binding" refers to the binding of the antibody to an epitope of the antigen(either human VEGF or human ANG-2) in an in vitro assay, preferably in an plasmon resonance assay (BIAcore, GE-Healthcare Uppsala, Sweden) (Example 2) with purified wild-type antigen. The affinity of the binding is defined by the terms ka (rate constant for the association of the antibody from the antibody/antigen complex), k_D (dissociation constant), and K_D (k_D/ka). Binding or specifically binding means a binding affinity (K_D) of 10^{-8} mol/l or less, preferably 10^{-9} M to 10^{-13} mol/l.

[0057] Binding of the antibody to the FcγRIII can be investigated by a BIAcore assay (GE-Healthcare Uppsala, Sweden). The affinity of the binding is defined by the terms ka (rate constant for the association of the antibody from the antibody/antigen complex), k_D (dissociation constant), and K_D (k_D/ka).

[0058] As used herein, the term "not binding to ANG-1" or "not specifically binding to ANG-1" denotes that the antibody has an EC50-value above 8000 ng/ml in an in-vitro ANG-1 binding ELISA assay (according to Example 9).

[0059] The term "epitope" includes any polypeptide determinant capable of specific binding to an antibody. In certain embodiments, epitope determinant include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody.

[0060] In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules.

[0061] In one embodiment of the invention the bispecific antibody comprises a full length parent antibody as scaffold.

[0062] The term "full length antibody" denotes an antibody consisting of two "full length antibody heavy chains" and two "full length antibody light chains" A "full length antibody heavy chain" is a polypeptide consisting in N-terminal to C-terminal direction of an antibody heavy chain variable domain (VH), an antibody constant heavy chain domain 1 (CH1), an antibody hinge region (HR), an antibody heavy chain constant domain 2 (CH2), and an antibody heavy chain constant domain 3 (CH3), abbreviated as VH-CH1-HR-CH2-CH3; and optionally an antibody heavy chain constant domain 4 (CH4) in case of an antibody of the subclass IgE. Preferably the "full length antibody heavy chain" is a polypeptide consisting in N-terminal to C-terminal direction of VH, CH1, HR, CH2 and CH3. A "full length antibody light chain" is a polypeptide consisting in N-terminal to C-terminal direction of an antibody light chain variable domain (VL), and an antibody light chain constant domain (CL), abbreviated as VL-CL. The antibody light chain constant domain (CL) can be κ (kappa) or λ (lambda). The two full length antibody chains are linked together via inter-polypeptide disulfide bonds between the CL domain and the CH1 domain and between the hinge regions of the full length antibody heavy chains. Examples of typical full length antibodies are natural antibodies like IgG (e.g. IgG 1 and IgG2), IgM, IgA, IgD, and IgE.

[0063] Thus a monospecific bivalent (= full length) antibody comprising a first antigen-binding site and consisting of two antibody light chains and two antibody heavy chains is a full length antibody. The C-terminus of the heavy or light chain of said full length antibody denotes the last amino acid at the C-terminus of said heavy or light chain. The N-terminus of the heavy or light chain of said full length antibody denotes the last amino acid at the N- terminus of said heavy or light chain.

[0064] The bispecific antibodies specifically binding to human vascular endothelial growth factor (VEGF) and human angiopoietin-2 (ANG-2) according to the invention are bivalent antibodies with two different specifities as e.g. a) described in WO 2009/080251, WO 2009/080252 or WO 2009/080253 (domain exchanged antibodies- see Example 13) or b) based on a scFab-Fc fusion antibody wherein one single chain Fab fragment (eventually disulfide stabilized) is specific for VEGF and the other single chain Fab fragment (eventually disulfide stabilized) for ANG-2 (see Example 14) or c) described in Ridgway, J.B., Protein Eng. 9 (1996) 617-621; WO 96/027011; Merchant, A.M., et al., Nature Biotech 16 (1998) 677-681; Atwell, S., et al., J. Mol. Biol. 270 (1997) 26-35 and EP 1 870 459A1.

[0065] In one embodiment the bispecific antibody according to the invention is characterized in comprising as amino acid sequences of SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123 and SEQ ID NO: 124 or variants thereof.

[0066] In one embodiment the bispecific antibody according to the invention is characterized in comprising as amino acid sequences of SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127 and SEQ ID NO: 128 or variants thereof.

[0067] In one embodiment the bispecific antibody according to the invention is characterized in comprising as amino acid sequences of SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131 and SEQ ID NO: 132 or variants thereof.

[0068] In one embodiment the bispecific antibody according to the invention is characterized in comprising as amino acid sequences of SEQ ID NO: 133, and SEQ ID NO: 134 or variants thereof.

[0069] In one embodiment the bispecific antibody according to the invention is characterized in comprising as amino acid sequences of SEQ ID NO: 135, and SEQ ID NO: 136 or variants thereof.

[0070] These amino acid sequences are based on the heavy chain variable domains of SEQ ID NO: 7, and the light chain variable domains of SEQ ID NO: 8 (derived from bevacizumab (Avastin)) as first antigen-binding site binding to VEGF, and on the heavy chain variable domains of SEQ ID NO: 52, and the light chain variable domains of SEQ ID NO: 53 (derived from Ang2i_LC06)) as second antigen-binding site binding to ANG-2.

[0071] The binding sites in an antibody according to the invention are each formed by a pair of two variable domains, i.e. of one heavy chain variable domain and one light chain variable domain. The minimal binding site determinant in an antibody is the heavy chain CDR3 region.

[0072] In a further embodiment said bispecific antibody is characterized in that the constant region derived of human origin.

[0073] In a further embodiment said bispecific antibody is characterized in that the constant region of the bispecific antibody according to the invention is of human IgG1 subclass, or of human IgG1 subclass with the mutations L234A and L235A.

[0074] In a further embodiment said bispecific antibody is characterized in that the constant region of the bispecific antibody according to the invention antibody is of human IgG2 subclass.

[0075] In a further embodiment said bispecific antibody is characterized in that the constant region of the bispecific antibody according to the invention antibody is of human IgG3 subclass.

[0076] In a further embodiment said bispecific antibody is characterized in the constant region of the bispecific antibody according to the invention is of human IgG4 subclass or, of human IgG4 subclass with the additional mutation S228P.

[0077] It has now been found that the bispecific antibodies against human VEGF and human ANG-2 according to the current invention have improved characteristics such as biological or pharmacological activity, pharmacokinetic properties or toxicity.They show increased in vivo tumor growth inhibition and/or inhibition of tumor angiogenesis when compared

to the monospecific parent antibodies against VEGF and ANG-2 (see Examples 16, 17 and 18: comparison of different bispecific <VEGF-ANG-2> antibodies bevacizumab-ANG2i-LC06 with the monospecific antibodies Avastin (bevacicumab) alone, ANG21-LC06 alone, or both in combination).

[0078] Furthermore less toxic side effects (which is reflected in the improved body weight of the test animals as well as less deaths of test animals during the in vivo application) compared to the application of two corresponding individual monospecific antibodies against VEGF and ANG-2 in combination also represent an advantage of the bispecific antibodies according to the invention.

[0079] Furthermore the bispecific antibodies according to the current invention may provide benefits such as reduced dose and/or frequency of administration and concomitantly cost savings.

[0080] The term "constant region" as used within the current applications denotes the sum of the domains of an antibody other than the variable region. The constant region is not involved directly in binding of an antigen, but exhibits various effector functions. Depending on the amino acid sequence of the constant region of their heavy chains, antibodies are divided in the classes: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses, such as IgG1, IgG2, IgG3, and IgG4, IgA1 and IgA2. The heavy chain constant regions that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The light chain constant regions which can be found in all five antibody classes are called κ (kappa) and λ (lambda).

[0081] The term "constant region derived from human origin" as used in the current application denotes a constant heavy chain region of a human antibody of the subclass IgG1, IgG2, IgG3, or IgG4 and/or a constant light chain kappa or lambda region. Such constant regions are well known in the state of the art and e.g. described by Kabat, E.A., (see e.g. Johnson, G., and Wu, T.T., Nucleic Acids Res. 28 (2000) 214-218; Kabat, E.A., et al., Proc. Natl. Acad. Sci. USA 72 (1975) 2785-2788).

[0082] While antibodies of the IgG4 subclass show reduced Fc receptor (FcγRIIIa) binding, antibodies of other IgG subclasses show strong binding. However Pro238, Asp265, Asp270, Asn297 (loss of Fc carbohydrate), Pro329, Leu234, Leu235, Gly236, Gly237, Ile253, Ser254, Lys288, Thr307, Gln311, Asn434, and His435 are residues which, if altered, provide also reduced Fc receptor binding (Shields, R.L., et al., J. Biol. Chem. 276 (2001) 6591-6604; Lund, J., et al., FASEB J. 9 (1995) 115-119; Morgan, A., et al., Immunology 86 (1995) 319-324; EP 0 307 434).

[0083] In one embodiment an antibody according to the invention has a reduced FcR binding compared to an IgGI antibody and the monospecific bivalent (full length) parent antibody is in regard to FcR binding of IgG4 subclass or of IgG1 or IgG2 subclass with a mutation in S228, L234, L235 and/or D265, and/ or contains the PVA236 mutation. In one embodiment the mutations in the monospecific bivalent (full length) parent antibody are S228P, L234A, L235A, L235E and/or PVA236. In another embodiment the mutations in the monospecific bivalent (full length) parent antibody are in IgG4 S228P and in IgG1 L234A and L235A. Constant heavy chain regions are shown in SEQ ID NO: 35 and 36. In one embodiment the constant heavy chain region of the monospecific bivalent (full length) parent antibody is of SEQ ID NO: 35 with mutations L234A and L235A. In another embodiment the constant heavy chain region of the monospecific bivalent (full length) parent antibody is of SEQ ID NO: 36 with mutation S228P. In another embodiment the constant light chain region of the monospecific bivalent (full length) parent antibody is a kappa light chain region of SEQ ID NO: 37 or lambda light chain region of SEQ ID NO: 34. Preferably the constant heavy chain region of the monospecific bivalent (full length) parent antibody is of SEQ ID NO: 35 or of SEQ ID NO: 36 with mutation S228P.

[0084] The constant region of an antibody is directly involved in ADCC (antibody-dependent cell-mediated cytotoxicity) and CDC (complement-dependent cytotoxicity). Complement activation (CDC) is initiated by binding of complement factor Clq to the constant region of most IgG antibody subclasses. Binding of Clq to an antibody is caused by defined protein-protein interactions at the so called binding site. Such constant region binding sites are known in the state of the art and described e.g. by Lukas, T.J., et al., J. Immunol. 127 (1981) 2555-2560; Brunhouse, R. and Cebra, J.J., Mol. Immunol. 16 (1979) 907-917; Burton, D.R., et al., Nature 288 (1980) 338-344; Thommesen, J.E., et al., Mol. Immunol. 37 (2000) 995-1004; Idusogie, E.E., et al., J. Immunol. 164 (2000) 4178-4184; Hezareh, M., et al., J. Virol. 75 (2001) 12161-12168; Morgan, A., et al., Immunology 86 (1995) 319-324; and EP 0 307 434. Such constant region binding sites are, e.g., characterized by the amino acids L234, L235, D270, N297, E318, K320, K322, P331, and P329 (numbering according to EU index of Kabat).

[0085] The term "antibody-dependent cellular cytotoxicity (ADCC)" refers to lysis of human target cells by an antibody according to the invention in the presence of effector cells. ADCC is measured preferably by the treatment of a preparation of CCR5 expressing cells with an antibody according to the invention in the presence of effector cells such as freshly isolated PBMC or purified effector cells from buffy coats, like monocytes or natural killer (NK) cells or a permanently growing NK cell line.

[0086] The term "complement-dependent cytotoxicity (CDC)" denotes a process initiated by binding of complement factor C1q to the Fc part of most IgG antibody subclasses. Binding of C1q to an antibody is caused by defined protein-protein interactions at the so called binding site. Such Fc part binding sites are known in the state of the art (see above). Such Fc part binding sites are, e.g., characterized by the amino acids L234, L235, D270, N297, E318, K320, K322, P331, and P329 (numbering according to EU index of Kabat). Antibodies of subclass IgG1, IgG2, and IgG3 usually show

complement activation including C1q and C3 binding, whereas IgG4 does not activate the complement system and does not bind C1q and/or C3.

[0087] The antibody according to the invention is produced by recombinant means. Thus, one aspect of the current invention is a nucleic acid encoding the antibody according to the invention and a further aspect is a cell comprising said nucleic acid encoding an antibody according to the invention. Methods for recombinant production are widely known in the state of the art and comprise protein expression in prokaryotic and eukaryotic cells with subsequent isolation of the antibody and usually purification to a pharmaceutically acceptable purity. For the expression of the antibodies as aforementioned in a host cell, nucleic acids encoding the respective modified light and heavy chains are inserted into expression vectors by standard methods. Expression is performed in appropriate prokaryotic or eukaryotic host cells like CHO cells, NS0 cells, SP2/0 cells, HEK293 cells, COS cells, PER.C6 cells, yeast, or E.coli cells, and the antibody is recovered from the cells (supernatant or cells after lysis). General methods for recombinant production of antibodies are well-known in the state of the art and described, for example, in the review articles of Makrides, S.C., Protein Expr. Purif. 17 (1999) 183-202; Geisse, S., et al., Protein Expr. Purif. 8 (1996) 271-282; Kaufman, R.J., Mol. Biotechnol. 16 (2000) 151-161; Werner, R.G., Drug Res. 48 (1998) 870-880.

[0088] The bispecific antibodies are suitably separated from the culture medium by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography. DNA and RNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures. The hybridoma cells can serve as a source of such DNA and RNA. Once isolated, the DNA may be inserted into expression vectors, which are then transfected into host cells such as HEK 293 cells, CHO cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of recombinant monoclonal antibodies in the host cells.

[0089] Amino acid sequence variants (or mutants) of the bispecific antibody are prepared by introducing appropriate nucleotide changes into the antibody DNA, or by nucleotide synthesis. Such modifications can be performed, however, only in a very limited range, e.g. as described above. For example, the modifications do not alter the above mentioned antibody characteristics such as the IgG isotype and antigen binding, but may improve the yield of the recombinant production, protein stability or facilitate the purification.

[0090] The term "host cell" as used in the current application denotes any kind of cellular system which can be engineered to generate the antibodies according to the current invention. In one embodiment HEK293 cells and CHO cells are used as host cells. As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological activity as screened for in the originally transformed cell are included.

[0091] Expression in NS0 cells is described by, e.g., Barnes, L.M., et al., Cytotechnology 32 (2000) 109-123; Barnes, L.M., et al., Biotech. Bioeng. 73 (2001) 261-270. Transient expression is described by, e.g., Durocher, Y., et al., Nucl. Acids. Res. 30 (2002) E9. Cloning of variable domains is described by Orlandi, R., et al., Proc. Natl. Acad. Sci. USA 86 (1989) 3833-3837; Carter, P., et al., Proc. Natl. Acad. Sci. USA 89 (1992) 4285-4289; and Norderhaug, L., et al., J. Immunol. Methods 204 (1997) 77-87. A preferred transient expression system (HEK 293) is described by Schlaeger, E.-J., and Christensen, K., in Cytotechnology 30 (1999) 71-83 and by Schlaeger, E.-J., in J. Immunol. Methods 194 (1996) 191-199.

[0092] The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, enhancers and polyadenylation signals.

[0093] A nucleic acid is "operably linked" when it is placed in a functional relationship with another nucleic acid sequence. For example, DNA for a pre-sequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a pre-protein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0094] Purification of antibodies is performed in order to eliminate cellular components or other contaminants, e.g. other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis, and others well known in the art. See Ausubel, F., et al., ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987). Different methods are well established and widespread used for protein purification, such as affinity chromatography with microbial proteins (e.g. protein A or protein G affinity chromatography), ion exchange chromatography (e.g. cation exchange (carboxymethyl resins), anion exchange (amino ethyl resins) and mixed-mode exchange), thiophilic adsorption (e.g. with beta-mercap-

toethanol and other SH ligands), hydrophobic interaction or aromatic adsorption chromatography (e.g. with phenyl-sepharose, aza-arenophilic resins, or m-aminophenylboronic acid), metal chelate affinity chromatography (e.g. with Ni (II)- and Cu(II)-affinity material), size exclusion chromatography, and electrophoretical methods (such as gel electrophoresis, capillary electrophoresis) (Vijayalakshmi, M.A., Appl. Biochem. Biotech. 75 (1998) 93-102).

[0095] One aspect of the invention is a pharmaceutical composition comprising an antibody according to the invention. Another aspect of the invention is the use of an antibody according to the invention for the manufacture of a pharmaceutical composition. A further aspect of the invention is a method for the manufacture of a pharmaceutical composition comprising an antibody according to the invention. In another aspect, the present invention provides a composition, e.g. a pharmaceutical composition, containing an antibody according to the present invention, formulated together with a pharmaceutical carrier.

[0096] One embodiment of the invention is the bispecific antibody according to the invention for the treatment of cancer.

[0097] Another aspect of the invention is said pharmaceutical composition for the treatment of cancer.

[0098] Another aspect of the invention is the use of an antibody according to the invention for the manufacture of a medicament for the treatment of cancer.

[0099] Another aspect of the invention is method of treatment of patient suffering from cancer by administering an antibody according to the invention to a patient in the need of such treatment.

[0100] As used herein, "pharmaceutical carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g. by injection or infusion).

[0101] A composition of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. To administer a compound of the invention by certain routes of administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Pharmaceutical carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art.

[0102] The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intra-articular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

[0103] The term cancer as used herein refers to proliferative diseases, such as lymphomas, lymphocytic leukemias, lung cancer, non small cell lung (NSCL) cancer, bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwanomas, ependymonas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma and Ewings sarcoma, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers.

[0104] Another aspect of the invention is the bispecific antibody according to the invention or said pharmaceutical composition as anti-angiogenic agent. Such anti-angiogenic agent can be used for the treatment of cancer, especially solid tumors, and other vascular diseases.

[0105] One embodiment of the invention is the bispecific antibody according to the invention for the treatment of vascular diseases.

[0106] Another aspect of the invention is said pharmaceutical composition for the treatment of of vascular diseases.

[0107] Another aspect of the invention is the use of an antibody according to the invention for the manufacture of a medicament for the treatment of vascular diseases.

[0108] Another aspect of the invention is method of treatment of patient suffering from vascular diseases by administering an antibody according to the invention to a patient in the need of such treatment.

[0109] The term "vascular diseases" includes Cancer, Inflammatory diseases, Atherosclerosis, Ischemia, Trauma, Sepsis, COPD, Asthma, Diabetes, AMD, Retinopathy, Stroke, Adipositas, Acute lung injury, Hemorrhage, Vascular leak e.g. Cytokine induced, Allergy, Graves' Disease, Hashimoto's Autoimmune Thyroiditis, Idiopathic Thrombocytopenic

Purpura, Giant Cell Arteritis, Rheumatoid Arthritis, Systemic Lupus Erythematosus (SLE), Lupus Nephritis, Crohn's Disease, Multiple Sclerosis, Ulcerative Colitis, especially to solid tumors, intraocular neovascular syndromes such as proliferative retinopathies or age-related macular degeneration (AMD), rheumatoid arthritis, and psoriasis (Folkman, J., et al., J. Biol. Chem. 267 (1992) 10931-10934; Klagsbrun, M., et al., Annu. Rev. Physiol. 53 (1991) 217-239; and Garner, A., Vascular diseases, In: Pathobiology of ocular disease, A dynamic approach, Garner, A., and Klintworth, G.K., (eds.), 2nd edition, Marcel Dekker, New York (1994), pp 1625-1710).

[0110] These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[0111] Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

[0112] Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[0113] The composition must be sterile and fluid to the extent that the composition is deliverable by syringe. In addition to water, the carrier preferably is an isotonic buffered saline solution.

[0114] Proper fluidity can be maintained, for example, by use of coating such as lecithin, by maintenance of required particle size in the case of dispersion and by use of surfactants. In many cases, it is preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, and sodium chloride in the composition.

[0115] As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

[0116] The term "transformation" as used herein refers to process of transfer of a vectors/nucleic acid into a host cell. If cells without formidable cell wall barriers are used as host cells, transfection is carried out e.g. by the calcium phosphate precipitation method as described by Graham, F.L., van der Eb, A.J., Virology 52 (1978) 546ff. However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used. If prokaryotic cells or cells which contain substantial cell wall constructions are used, e.g. one method of transfection is calcium treatment using calcium chloride as described by Cohen, S.N., et al., PNAS. 69 (1972) 2110-2114.

[0117] As used herein, "expression" refers to the process by which a nucleic acid is transcribed into mRNA and/or to the process by which the transcribed mRNA (also referred to as transcript) is subsequently being translated into peptides, polypeptides, or proteins. The transcripts and the encoded polypeptides are collectively referred to as gene product. If the polynucleotide is derived from genomic DNA, expression in a eukaryotic cell may include splicing of the mRNA. A "vector" is a nucleic acid molecule, in particular self-replicating, which transfers an inserted nucleic acid molecule into and/or between host cells. The term includes vectors that function primarily for insertion of DNA or RNA into a cell (e.g., chromosomal integration), replication of vectors that function primarily for the replication of DNA or RNA, and expression vectors that function for transcription and/or translation of the DNA or RNA. Also included are vectors that provide more than one of the functions as described.

[0118] An "expression vector" is a polynucleotide which, when introduced into an appropriate host cell, can be transcribed and translated into a polypeptide. An "expression system" usually refers to a suitable host cell comprised of an expression vector that can function to yield a desired expression product.

Description of the Amino acid Sequences

[0119]

SEQ ID NO:	1	heavy chain CDR3, <VEGF>bevacizumab
SEQ ID NO:	2	heavy chain CDR2, <VEGF>bevacizumab
SEQ ID NO:	3	heavy chain CDR1, <VEGF>bevacizumab
SEQ ID NO:	4	light chain CDR3, <VEGF>bevacizumab
SEQ ID NO:	5	light chain CDR2, <VEGF>bevacizumab
SEQ ID NO:	6	light chain CDR1, <VEGF>bevacizumab
SEQ ID NO:	7	heavy chain variable domain, <VEGF>bevacizumab
SEQ ID NO:	8	light, chain variable domain, <VEGF>bevacizumab
SEQ ID NO:	9	heavy chain CDR3, <VEGF>ranibizumab
SEQ ID NO:	10	heavy chain CDR2, <VEGF>ranibizumab
SEQ ID NO:	11	heavy chain CDR1, <VEGF>ranibizumab
SEQ ID NO:	12	light chain CDR3, <VEGF>ranibizumab
SEQ ID NO:	13	light chain CDR2, <VEGF>ranibizumab
SEQ ID NO:	14	light chain CDR1, <VEGF>ranibizumab
SEQ ID NO:	15	heavy chain variable domain, <VEGF>ranibizumab
SEQ ID NO:	16	light chain variable domain, <VEGF>ranibizumab
SEQ ID NO:	17	heavy chain CDR3, <VEGF>HuMab G6-31
SEQ ID NO:	18	heavy chain CDR2, <VEGF> HuMab G6-31
SEQ ID NO:	19	heavy chain CDR1, <VEGF> HuMab G6-31
SEQ ID NO:	20	light chain CDR3, <VEGF> HuMab G6-31
SEQ ID NO:	21	light chain CDR2, <VEGF> HuMab G6-31
SEQ ID NO:	22	light chain CDR1, <VEGF> HuMab G6-31
SEQ ID NO:	23	heavy chain variable domain, <VEGF> HuMab G6-31
SEQ ID NO:	24	light chain variable domain, <VEGF> HuMab G6-31
SEQ ID NO:	25	heavy chain CDR3, <ANG-2> Mab 536
SEQ ID NO:	26	heavy chain CDR2, <ANG-2> Mab 536
SEQ ID NO:	27	heavy chain CDR1, <ANG-2> Mab 536
SEQ ID NO:	28	light chain CDR3, <ANG-2> Mab 536
SEQ ID NO:	29	light chain CDR2, <ANG-2> Mab 536
SEQ ID NO:	30	light chain CDR1, <ANG-2> Mab 536
SEQ ID NO:	31	heavy chain variable domain, <ANG-2> Mab 536
SEQ ID NO:	32	light chain variable domain, <ANG-2> Mab 536
SEQ ID NO:	33	(G4S)4 linker
SEQ ID NO:	34	lambda light chain constant region
SEQ ID NO:	35	human heavy chain constant region derived from IgG1
SEQ ID NO:	36	human heavy chain constant region derived from IgG4
SEQ ID NO:	37	kappa light chain constant region
SEQ ID NO:	38	heavy chain CDR3, <ANG-2> Ang2s_R3_LC03
SEQ ID NO:	39	heavy chain CDR2, <ANG-2> Ang2s_R3_LC03

(continued)

SEQ ID NO:	40	heavy chain CDR1, <ANG-2> Ang2s_R3_LC03
SEQ ID NO:	41	light chain CDR3, <ANG-2> Ang2s_R3_LC03
SEQ ID NO:	42	light chain CDR2, <ANG-2> Ang2s_R3_LC03
SEQ ID NO:	43	light chain CDR1, <ANG-2> Ang2s_R3_LC03
SEQ ID NO:	44	heavy chain variable domain, <ANG-2> Ang2s_R3_LC03
SEQ ID NO:	45	light chain variable domain, <ANG-2> Ang2s_R3_LC03
SEQ ID NO:	46	heavy chain CDR3, <ANG-2>Ang2i_LC06
SEQ ID NO:	47	heavy chain CDR2, <ANG-2> Ang2i_LC06
SEQ ID NO:	48	heavy chain CDR1, <ANG-2>Ang2i_LC06
SEQ ID NO:	49	light chain CDR3, <ANG-2>Ang2i_LC06
SEQ ID NO:	50	light chain CDR2, <ANG-2>Ang2i_LC06
SEQ ID NO:	51	light chain CDR1, <ANG-2>Ang2i_LC06
SEQ ID NO:	52	heavy chain variable domain, <ANG-2>Ang2i_LC06
SEQ ID NO:	53	light chain variable domain, <ANG-2>Ang2i_LC06
SEQ ID NO:	54	heavy chain CDR3, <ANG-2>Ang2i_LC07
SEQ ID NO:	55	heavy chain CDR2, <ANG-2>Ang2i_LC07
SEQ ID NO:	56	heavy chain CDR1, <ANG-2>Ang2i_LC07
SEQ ID NO:	57	light chain CDR3, <ANG-2>Ang2i_LC07
SEQ ID NO:	58	light chain CDR2, <ANG-2>Ang2i_LC07
SEQ ID NO:	59	light chain CDR1, <ANG-2>Ang2i_LC07
SEQ ID NO:	60	heavy chain variable domain, <ANG-2>Ang2i_LC07
SEQ ID NO:	61	light chain variable domain, <ANG-2>Ang2i_LC07
SEQ ID NO:	62	heavy chain CDR3, <ANG-2>Ang2k_LC08
SEQ ID NO:	63	heavy chain CDR2, <ANG-2> Ang2k_LC08
SEQ ID NO:	64	heavy chain CDR1, <ANG-2> Ang2k_LC08
SEQ ID NO:	65	light chain CDR3, <ANG-2> Ang2k_LC08
SEQ ID NO:	66	light chain CDR2, <ANG-2> Ang2k_LC08
SEQ ID NO:	67	light chain CDR1, <ANG-2> Ang2k_LC08
SEQ ID NO:	68	heavy chain variable domain, <ANG-2> Ang2k_LC08
SEQ ID NO:	69	light chain variable domain, <ANG-2> Ang2k_LC08
SEQ ID NO:	70	heavy chain CDR3, <ANG-2> Ang2s_LC09
SEQ ID NO:	71	heavy chain CDR2, <ANG-2> Ang2s_LC09
SEQ ID NO:	72	heavy chain CDR1, <ANG-2> Ang2s_LC09
SEQ ID NO:	73	light chain CDR3, <ANG-2> Ang2s_LC09
SEQ ID NO:	74	light chain CDR2, <ANG-2> Ang2s_LC09
SEQ ID NO:	75	light chain CDR1, <ANG-2> Ang2s_LC09
SEQ ID NO:	76	heavy chain variable domain, <ANG-2> Ang2s_LC09
SEQ ID NO:	77	light chain variable domain, <ANG-2> Ang2s_LC09
SEQ ID NO:	78	heavy chain CDR3, <ANG-2> Ang2i_LC10

(continued)

SEQ ID NO:	79	heavy chain CDR2, <ANG-2> Ang2i_LC10
SEQ ID NO:	80	heavy chain CDR1, <ANG-2> Ang2i_LC10
SEQ ID NO:	81	light chain CDR3, <ANG-2> Ang2i_LC10
SEQ ID NO:	82	light chain CDR2, <ANG-2> Ang2i_LC10
SEQ ID NO:	83	light chain CDR1, <ANG-2> Ang2i_LC10
SEQ ID NO:	84	heavy chain variable domain, <ANG-2> Ang2i_LC10
SEQ ID NO:	85	light chain variable domain, <ANG-2> Ang2i_LC10
SEQ ID NO:	86	heavy chain CDR3, <ANG-2> Ang2k_LC11
SEQ ID NO:	87	heavy chain CDR2, <ANG-2> Ang2k_LC11
SEQ ID NO:	88	heavy chain CDR1, <ANG-2> Ang2k_LC11
SEQ ID NO:	89	light chain CDR3, <ANG-2> Ang2k_LC11
SEQ ID NO:	90	light chain CDR2, <ANG-2> Ang2k_LC11
SEQ ID NO:	91	light chain CDR1, <ANG-2> Ang2k_LC11
SEQ ID NO:	92	heavy chain variable domain, <ANG-2> Ang2k_LC11
SEQ ID NO:	93	light chain variable domain, <ANG-2> Ang2k_LC11
SEQ ID NO:	94	heavy chain CDR3, <VEGF>B20-4.1
SEQ ID NO:	95	heavy chain CDR2, <VEGF>B20-4.1
SEQ ID NO:	96	heavy chain CDR1, <VEGF>B20-4.1
SEQ ID NO:	97	light chain CDR3, <VEGF>B20-4.1
SEQ ID NO:	98	light chain CDR2, <VEGF>B20-4.1
SEQ ID NO:	99	light chain CDR1, <VEGF>B20-4.1
SEQ ID NO:	100	heavy chain variable domain, <VEGF>B20-4.1
SEQ ID NO:	101	light chain variable domain, <VEGF>B20-4.1
SEQ ID NO:	102	bevacizumab heavy chain Ang2i_LC06 scFv fusion peptide of <VEGF-ANG-2> TvAb-2441-bevacizumab-LC06
SEQ ID NO:	103	bevacizumab heavy chain Ang2i_LC08 scFv fusion peptide of <VEGF-ANG-2> TvAb-2441-bevacizumab-LC08
SEQ ID NO:	104	light chain of bevacizumab
SEQ ID NO:	105	Human vascular endothelial growth factor (VEGF)
SEQ ID NO:	106	Human angiopoietin-2 (ANG-2)
SEQ ID NO:	107	Human angiopoietin-1 (ANG-1)
SEQ ID NO:	108	Human Tie-2 receptor
SEQ ID NO:	109	Heavy chain 1 of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFAb-Avastin-LC06-2620
SEQ ID NO:	110	Light chain of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFAb-Avastin-LC06-2620
SEQ ID NO:	111	Heavy chain 1 of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFab-Avastin-Ang2i-LC06-2640
SEQ ID NO:	112	Light chain of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFab-Avastin-Ang2i-LC06-2640

(continued)

SEQ ID NO:	113	Heavy chain 1 of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFab-Avastin-Ang2i-LC06-2641
SEQ ID NO:	114	Light chain of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFab-Avastin-Ang2i-LC06-2641
SEQ ID NO:	115	Heavy chain 1 of bispecific, trivalent single chain Fab <VEGF-ANG-2> antibody molecule Avastin-LC06-KiH-C-scFab
SEQ ID NO:	116	Heavy chain 2 of bispecific, trivalent single chain Fab <VEGF-ANG-2> antibody molecule Avastin-LC06-KiH-C-scFab
SEQ ID NO:	117	Light chain of bispecific, trivalent single chain Fab <VEGF-ANG-2> antibody molecule Avastin-LC06-KiH-C-scFab
SEQ ID NO:	118	Heavy chain 1 of bispecific, trivalent <VEGF-ANG-2> antibody molecule Avastin-LC06-C-Fab-6CSS
SEQ ID NO:	119	Heavy chain 2 of bispecific, trivalent <VEGF-ANG-2> antibody molecule Avastin-LC06-C-Fab-6CSS
SEQ ID NO:	120	Light chain of bispecific, trivalent <VEGF-ANG-2> antibody molecule Avastin-LC06-C-Fab-6CSS
SEQ ID NO:	121	Heavy chain I of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-CH1-CL
SEQ ID NO:	122	Heavy chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-CH1-CL
SEQ ID NO:	123	Light chain 1 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-CH1-CL
SEQ ID NO:	124	Light chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-CH1-CL
SEQ ID NO:	125	Heavy chain 1 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-VH-VL
SEQ ID NO:	126	Heavy chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-VH-VL
SEQ ID NO:	127	Light chain 1 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06- VH-VL
SEQ ID NO:	128	Light chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06- VH-VL
SEQ ID NO:	129	Heavy chain 1 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-VH-VL-SS
SEQ ID NO:	130	Heavy chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-VH-VL-SS
SEQ ID NO:	131	Light chain 1 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06- VH-VL-SS
SEQ ID NO:	132	Light chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06- VH-VL-SS
SEQ ID NO:	133	Heavy chain 1 ofbispecific, bivalent ScFab-Fc fusion <VEGF-ANG-2> antibody molecule Avastin-LC06-N-scFab
SEQ ID NO:	134	Heavy chain 2 of bispecific, bivalent ScFab-Fc fusion <VEGF-ANG-2> antibody molecule Avastin-LC06-N-scFab
SEQ ID NO:	135	Heavy chain 1 of bispecific, bivalent ScFab-Fc fusion <VEGF-ANG-2> antibody molecule Avastin-LC06-N-scFabSS

(continued)

SEQ ID NO:	136	Heavy chain 2 of bispecific, bivalent ScFab-Fc fusion <VEGF-ANG-2> antibody molecule Avastin-LC06-N-scFabSS

[0120] The following examples, sequence listing and figures are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

Description of the Figures

[0121]

Figure 1A Schematic structure of one tetravalent embodiment of a bispecific antibody according to the invention binding to VEGF and ANG-2, wherein one of the Antigens A or B is VEGF, while the other is ANG-2. The structure is based on a full length antibody binding to Antigen A, to which two (optionally disulfide-stabilized) single chain Fv' s binding to Antigen B, are linked via the a peptide-linker.

Figure 1B Schematic representation of the generated bispecific tetravalent antibodies using the TvAb nomenclature (see Examples) - either without or with disulfide stabilization of the scFv

Figure 2A Schematic representation of disulfide-stabilized <VEGF-ANG-2> bispecific tetravalent antibody (=<VEGF-ANG-2> TvAb6; No. 2331, see Table 3)

Figure 2B Plasmid maps of the modified heavy chain and the light vectors used for the expression of disulfide-stabilized <VEGF-ANG-2> TvAb6

Figure 3 SDS-PAGE of purified disulfide-stabilized <VEGF-ANG-2> TvAb6 in comparison to the "standard" human IgG1 antibody G6-31 (<VEGF> HuMab G6-31) under reducing and non-reducing conditions

Figure 4 Size exclusion chromatography of purified disulfide-stabilized <VEGF-ANG-2> TvAb6 in comparison to the "standard" human IgG1 antibody G6-31 shows that disulfide-stabilized TvAb6 does not form again aggregates upon purification

Figure 5 Schematic view and results from VEGF binding ELISA. disulfide-stabilized <VEGF-ANG-2> TvAb6 binds to VEGF comparable to <VEGF> G6-31. <ANG-2> Mab536 does not bind to VEGF

Figure 6A Schematic view and results from ANG-2 binding ELISA. disulfide-stabilized <VEGF-ANG-2> TvAb6 binds to ANG-2 comparable to <ANG-2> Mab536. <VEGF> G6-31 does not bind to ANG-2.

Figure 6B Schematic view and results from ANG-2 binding analysis by surface plasmon resonance (Biacore). disulfide-stabilized <VEGF-ANG-2> TvAb6 binds to ANG-2 with comparable affinity as <ANG-2> Mab536.

Figure 7 Schematic view and results from VEGF-ANG-2 bridging ELISA. disulfide-stabilized <VEGF-ANG-2> TvAb6 binds simultaneously to VEGF and ANG-2 whereas <VEGF> G6-31 and <ANG-2> Mab536 are not capable of binding simultaneously to VEGF and ANG-2.

Figure 8a Efficacy of disulfide-stabilized <VEGF-ANG-2> TvAb6 in comparison to <ANG-2> Mab536, <VEGF> G6-31 and the combination of Mab536 and G6-31 in the staged subcutaneous Colo205 xenograft model in Scid beige mice (study ANG2_Pz_Colo205_003)

Figure 8b Efficacy of disulfide-stabilized, <VEGF-ANG-2> TvAb6 in comparison to <ANG-2> Mab536, <VEGF> G6-31 and the combination of Mab536 and G6-31 in the staged subcutaneous Colo205 xenograft model in Scid beige mice (study ANG2_Pz_Colo205_005)

Figure 9 Blocking of VEGF-induced tube formation by the bispecific tetravalent antibody <VEGF-ANG-2> TvAb6- Results

Figure 10A + B Blocking of VEGF-induced tube formation by the b disulfide-stabilized <VEGF-ANG-2> TvAb6 -- Quantitative analysis

Figure 11 Schematic view of VEGF binding analysis by surface plasmon resonance (Biacore).

Figure 12 Kinetic characteristics of the two <VEGF> antibodies <VEGF-Ang-2> TvAb6 and <VEGF> G6-31 in a Ka-Kd plot.

Figure 13 Schematic view of surface plasmon resonance (Biacore) assay to detect simultaneous binding of ANGPT2 and VEGF to bispecific antibodies

Figure 14 Results from surface plasmon resonance (Biacore) experiments showing that TvAb6 binds simultaneously to ANGPT2 and VEGF.

Figure 15A + B A) Schematic representation of the bispecific and simultaneous Biacore binding assay of the <VEGF-ANG-2> bispecific antibodies. B) Biacore data demonstrating simultaneous binding of ANG-2 and

VEGF to TvAb-2441-bevacicumab_LC06

Figure 16A +B Tie2 phosphorylation of the bispecific antibodies <VEGF-ANG-2>TvAb-2441-bevacizumab-LC06 and <VEGF-ANG-2>TvAb-2441, in comparison with the anti-Ang2 antibodies <ANG-2>Ang2i_LC06 and < ANG-2>Ang2k_LC08

Figure 17 Schematic representation of human Angiopoietin interaction ELISA

Figure 18 VEGF-induced HUVEC proliferation of <VEGF-ANG-2>TvAb-2441-bevacizumab-LC06 and <VEGF-ANG-2>TvAb-2441-bevacizumab-LC08 and bevacizumab

Figure 19 In vivo anti-angiogenic efficacy of bispecific antibody <VEGF-ANG-2> bevacizumab-LC06 antibody in comparison to <ANG-2> ANG2i-LC06, and the combination of <ANG-2> ANG2i-LC06 and Avastin (bevacizumab) in Calu3 xenograft model monitored via labeled anti-CD31 antibody and the relative change of CD31 signal during therapy.

Experimental Procedure

Examples

Materials & general methods

[0122] General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991). Amino acids of antibody chains are numbered and referred to according to EU numbering (Edelman, G.M., et al., Proc. Natl. Acad. Sci. USA 63 (1969) 78-85; Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD, (1991)).

Recombinant DNA techniques

[0123] Standard methods were used to manipulate DNA as described in Sambrook, J. et al., Molecular cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. The molecular biological reagents were used according to the manufacturer's instructions.

Gene synthesis

[0124] Desired gene segments were prepared from oligonucleotides made by chemical synthesis, The gene segments, which are flanked by singular restriction endonuclease cleavage sites, were assembled by annealing and ligation of oligonucleotides including PCR amplification and subsequently cloned via the indicated restriction sites e.g. KpnI/ SacI or AscI/PacI into a pPCRScript (Stratagene) based pGA4 cloning vector. The DNA sequences of the subcloned gene fragments were confirmed by DNA sequencing. Gene synthesis fragments were ordered according to given specifcations at Geneart (Regensburg, Germany). All gene segments encoding light and heavy chains of Ang-2/VEGF bispecific antibodies were synthesized with a 5'-end DNA sequence coding for a leader peptide (MGWSCIILFLVATATGVHS), which targets proteins for secretion in eukaryotic cells, and 5'-BamHI and 3'-XbaI restriction sites. DNA sequences carrying disulfide stabilized "knobs-into-hole" modified heavy chains were designed with S354C and T366W mutations in the "knobs" heavy chain and Y349C, T366S, L368A and Y407V mutations in the "hole" heavy chain.

DNA sequence determination

[0125] DNA sequences were determined by double strand sequencing performed at MediGenomix GmbH (Martinsried, Germany) or Sequiserve GmbH (Vaterstetten, Germany).

DNA and protein sequence analysis and sequence data management

[0126] The GCG's (Genetics Computer Group, Madison, Wisconsin) software package version 10.2 and Infomax's Vector NT1 Advance suite version 8.0 was used for sequence creation, mapping, analysis, annotation and illustration.

Expression vectors (for Example 1) (reference)

[0127] For the expression of the described antibodies variants of expression plasmids for transient expression (e.g. in HEK293 EBNA or HEK293-F) cells or for stable expression (e.g. in CHO cells) based either on a cDNA organization with a CMV-Intron A promoter or on a genomic organization with a CMV promoter (e.g. Figure 2B) were applied.

[0128] Beside the antibody expression cassette the vectors contained:

- an origin of replication which allows replication of this plasmid in E. coli, and
- a ß-lactamase gene which confers ampicillin resistance in E. coli.

[0129] The transcription unit of the antibody gene is composed of the following elements:

- unique restriction site(s) at the 5' end
- the immediate early enhancer and promoter from the human cytomegalovirus,
- followed by the Intron A sequence in the case of the cDNA organization,
- a 5'-untranslated region of a human antibody gene,
- a immunoglobulin heavy chain signal sequence,
- the human antibody chain (heavy chain, modified heavy chain or light chain) either as cDNA or as genomic organization with an the immunoglobulin exon-intron organization
- a 3' untranslated region with a polyadenylation signal sequence, and
- unique restriction site(s) at the 3' end.

[0130] The fusion genes comprising the heavy chain sequences of the selected antibody and the C-terminal scFv fusion as described below were generated by PCR and/or gene synthesis and assembled with known recombinant methods and techniques by connection of the according nucleic acid segments e.g. using unique NsiI and EcoRI sites in the genomic heavy chain vectors. The subcloned nucleic acid sequences were verified by DNA sequencing. For transient and stable transfections larger quantities of the plasmids were prepared by plasmid preparation from transformed E. coli cultures (Nucleobond AX, Macherey-Nagel).

Expression vectors (for Example 10-14)

[0131] An expression vector was used which composed of the following elements:

- a hygromycin resistance gene as a selection marker,
- an origin of replication, oriP, of Epstein-Barr virus (EBV),
- an origin of replication from the vector pUC18 which allows replication of this plasmid in E. coli

- a beta-lactamase gene which confers ampicillin resistance in E. coli,
- the immediate early enhancer and promoter from the human cytomegalovirus (HCMV),
- the human 1-immunoglobulin polyadenylation ("poly A") signal sequence, and
- unique BamHI and XbaI restriction sites.

[0132] The immunoglobulin fusion genes comprising the heavy or light chain constucts as well as "knobs-into-hole" constructs with C-terminal VH and VL domains were prepared by gene synthesis and cloned into pGA 18 (ampR) plasmids as described. The pG18 (ampR) plasmids carrying the synthesized DNA segments and the Roche expression vector were digested with BamHI and XbaI restriction enzymes (Roche Molecular Biochemicals) and subjected to agarose gel electrophoresis. Purified heavy and light chain coding DNA segments were then ligated to the isolated Roche expression vector BamHI/XbaI fragment resulting in the final expression vectors. The final expression vectors were transformed into E. coli cells, expression plasmid DNA was isolated (Miniprep) and subjected to restriction enzyme analysis and DNA sequencing. Correct clones were grown in 150 ml LB-Amp medium, again plasmid DNA was isolated (Maxiprep) and sequence integrity confirmed by DNA sequencing.

Cell culture techniques

[0133] Standard cell culture techniques were used as described in Current Protocols in Cell Biology (2000), Bonifacino, J.S., Dasso, M., Harford, J.B., Lippincott-Schwartz, J. and Yamada, K.M. (eds.), John Wiley & Sons, Inc..

Transient transfections in HEK293-F system (for Example 1) (reference)

[0134] Antibodies were generated by transient transfection of the two plasmids encoding the heavy or modified heavy chain, respectively and the corresponding light chain using the HEK293-F system (Invitrogen) according to the manufacturer's instruction. Briefly, HEK293-F cells (Invitrogen) growing in suspension either in a shake flask or in a stirred fermenter in serumfree FreeStyle 293 expression medium (Invitrogen) were transfected with a mix of the two respective

expression plasmids and 293fectin or fectin (Invitrogen). For e.g. 2 L shake flask (Corning) HEK293-F cells were seeded at a density of 1.0E*6 cells/mL in 600 mL and incubated at 120 rpm, 8% CO2. The day after the cells were transfected at a cell density of ca. 1.5E*6 cells/mL with ca. 42 mL mix of A) 20 mL Opti-MEM (Invitrogen) with 600 µg total plasmid DNA (1 µg/mL) encoding the heavy or modified heavy chain, respectively and the corresponding light chain in an equimolar ratio and B) 20 ml Opti-MEM + 1.2 mL 293 fectin or fectin (2 µl/mL). According to the glucose consumption glucose solution was added during the course of the fermentation. The supernatant containing the secreted antibody was harvested after 5-10 days and antibodies were either directly purified from the supernatant or the supernatant was frozen and stored.

Transient transfections in HEK293-F system (for Example 10-14)

[0135] Recombinant immunoglobulin variants were expressed by transient transfection of human embryonic kidney 293-F cells using the FreeStyle™ 293 Expression System according to the manufacturer's instruction (Invitrogen, USA). Briefly, suspension FreeStyle™ 293-F cells were cultivated in FreeStyle™ 293 Expression medium at 37°C/8 % CO_2 and the cells were seeded in fresh medium at a density of 1-2x10^6 viable cells/ml on the day of transfection. DNA-293fectin™ complexes were prepared in Opti-MEM® I medium (Invitrogen, USA) using 325 µl of 293fectin™ (Invitrogen, Germany) and 250 µg of heavy and light chain plasmid DNA in a 1:1 molar ratio for a 250 ml final transfection volume. "Knobs-into-hole" DNA-293fectin complexes with two heavy chains and one light chain were prepared in Opti-MEM® I medium (Invitrogen, USA) using 325 µl of 293fectin™ (Invitrogen, Germany) and 250 µg of "Knobs-into-hole" heavy chain 1 and 2 and light chain plasmid DNA in a 1:1:2 molar ratio for a 250 ml final transfection volume. "Knobs-into-hole" DNA-293fectin complexes with two heavy chains were prepared in Opti-MEM® I medium (Invitrogen, USA) using 325 µl of 293fectin™ (Invitrogen, Germany) and 250 µg of "Knobs-into-hole" heavy chain 1 and 2 DNA in a 1:1 molar ratio for a 250 ml final transfection volume. CrossMab DNA-293fectin complexes were prepared in Opti-MEM® I medium (Invitrogen, USA) using 325 µl of 293fectin™ (Invitrogen, Germany) and 250 µg of "Knobs-into-hole" heavy chain 1 and 2 and light chain plasmid DNA in a 1:1:1:1 molar ratio for a 250 ml final transfection volume. Antibody containing cell culture supernatants were harvested 7 days after transfection by centrifugation at 14000 g for 30 minutes and filtered through a sterile filter (0.22 µm). Supernatants were stored at - 20° C until purification.

Protein determination

[0136] The protein concentration of purified antibodies and derivatives was determined by determining the optical density (OD) at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence according to Pace et. al., Protein Science, 1995, 4, 2411-1423.

Antibody concentration determination in supernatants

[0137] The concentration of antibodies and derivatives in cell culture supernatants was estimated by immunoprecipitation with Protein A Agarose-beads (Roche). 60 µL Protein A Agarose beads are washed three times in TBS-NP40 (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Nonidet-P40). Subsequently, 1 -15 mL cell culture supernatant are applied to the Protein A Agarose beads pre-equilibrated in TBS-NP40. After incubation for at 1 h at room temperature the beads are washed on an Ultrafree-MC-filter column (Amicon] once with 0.5 mL TBS-NP40, twice with 0.5 mL 2x phosphate buffered saline (2xPBS, Roche) and briefly four times with 0.5 mL 100 mM Na-citrate pH 5,0. Bound antibody is eluted by addition of 35 µl NuPAGE® LDS Sample Buffer (Invitrogen). Half of the sample is combined with NuPAGE® Sample Reducing Agent or left unreduced, respectively, and heated for 10 min at 70°C. Consequently, 20 µl are applied to an 4-12% NuPAGE® Bis-Tris SDS-PAGE (Invitrogen) (with MOPS buffer for non-reduced SDS-PAGE and MES buffer with NuPAGE® Antioxidant running buffer additive (Invitrogen) for reduced SDS-PAGE) and stained with Coomassie Blue.

[0138] The concentration of antibodies and derivatives in cell culture supernatants was measured by Protein A-HPLC chromatography. Briefly, cell culture supernatants containing antibodies and derivatives that bind to Protein A were applied to a HiTrap Protein A column (GE Healthcare) in 50 mM K2HPO4, 300 mM NaCl, pH 7.3 and eluted from the matrix with 550 mM acetic acid, pH 2.5 on a Dionex HPLC-System. The eluted protein was quantified by UV absorbance and integration of peak areas. A purified standard IgG1 antibody served as a standard.

[0139] Alternatively, the concentration of antibodies and derivatives in cell culture supernatants was measured by Sandwich-IgG-ELISA. Briefly, StreptaWell High Bind Strepatavidin A-96 well microtiter plates (Roche) were coated with 100 µL/well biotinylated anti-human IgG capture molecule F(ab')2<h-Fcgamma> BI (Dianova) at 0.1 µg/mL for 1 h at room temperature or alternatively over night at 4°C and subsequently washed three times with 200 µL/well PBS, 0.05% Tween (PBST, Sigma). 100 µL/well of a dilution series in PBS (Sigma) of the respective antibody containing cell culture supernatants was added to the wells and incubated for 1-2 h on a microtiterplate shaker at room temperature. The wells were washed three times with 200 µL/well PBST and bound antibody was detected with 100 µl F(ab')2<hFcgamma>POD

(Dianova) at 0.1 µg/mL as detection antibody for 1-2 h on a microtiterplate shaker at room temperature. Unbound detection antibody was washed away three times with 200 µL/well PBST and the bound detection antibody was detected by addition of 100 µL ABTS/well. Determination of absorbance was performed on a Tecan Fluor Spectrometer at a measurement wavelength of 405 nm (reference wavelength 492 nm).

Protein purification

[0140] Proteins were purified from filtered cell culture supernatants referring to standard protocols. In brief, antibodies were applied to a Protein A Sepharose column (GE Healthcare) and washed with PBS. Elution of antibodies was achieved at acidic pH followed by immediate neutralization of the sample. Aggregated protein was separated from monomeric antibodies by size exclusion chromatography (Superdex 200, GE Healthcare) in 20 mM Histidine, 140 mM NaCl pH 6.0. Monomeric antibody fractions were pooled, concentrated if required using e.g. a MILLIPORE Amicon Ultra (30 MWCO) centrifugal concentrator and stored at -80 °C. Part of the samples were provided for subsequent protein analytics and analytical characterization e.g. by SDS-PAGE, size exclusion chromatography, mass spectrometry and Endotoxin determination (see Figures 3 and 4).

SDS-PAGE

[0141] The NuPAGE® Pre-Cast gel system (Invitrogen) was used according to the manufacturer's instruction. In particular, 4-20 % NuPAGE® Novex® TRIS-Glycine Pre-Cast gels and a Novex® TRIS-Glycine SDS running buffer were used. (see e.g. Figure 3). Reducing of samples was achieved by adding NuPAGE® sample reducing agent prior to running the gel.

Analytical size exclusion chromatography

[0142] Size exclusion chromatography for the determination of the aggregation and oligomeric state of antibodies was performed by HPLC chromatography. Briefly, Protein A purified antibodies were applied to a Tosoh TSKgel G3000SW column in 300 mM NaCl, 50 mM KH2PO4/K2HPO4, pH 7.5 on an Agilent HPLC 1100 system or to a Superdex 200 column (GE Healthcare) in 2 x PBS on a Dionex HPLC-System. The eluted protein was quantified by UV absorbance and integration of peak areas. BioRad Gel Filtration Standard 151-1901 served as a standard. (see e.g. Figure 4).

Mass spectrometry

[0143] The total deglycosylated mass of crossover antibodies was determined and confirmed via electrospray ionization mass spectrometry (ESI-MS). Briefly, 100 µg purified antibodies were deglycosylated with 50 mU N-Glycosidase F (PNGaseF, ProZyme) in 100 mM KH2PO4/K2HPO4, pH 7 at 37°C for 12-24 h at a protein concentration of up to 2 mg/ml and subsequently desalted via HPLC on a Sephadex G25 column (GE Healthcare). The mass of the respective heavy and light chains was determined by ESI-MS after deglycosylation and reduction. In brief, 50 µg antibody in 115 µl were incubated with 60 µl 1M TCEP and 50 µl 8 M Guanidine-hydrochloride subsequently desalted. The total mass and the mass of the reduced heavy and light chains was determined via ESI-MS on a Q-Star Elite MS system equipped with a NanoMate source.

VEGF binding ELISA

[0144] The binding properties of the tetravalent antibodies (TvAb) was evaluated in an ELISA assay with full-length VEGF165-His protein (R&D Systems) (Figure 5). For this sake Falcon polystyrene clear enhanced microtiter plates were coated with 100 µl 2 µg/mL recombinant human VEGF165 (R&D Systems) in PBS for 2 h at room temperature or over night at 4°C. The wells were washed three times with 300µl PBST (0,2% Tween 20) and blocked with 200 µl 2% BSA 0,1% Tween 20 for 30 min at room temperature and subsequently washed three times with 300µl PBST. 100 µL/well of a dilution series (40 pM-0.01 pM) of purified <VEGF-ANG-2> TvAb and as a reference the human anti-ANG-2 antibody <ANG-2> antibody Mab536 (Oliner et al., Cancer Cell. 2004 Nov;6(5):507-16, US 2006/0122370) and the anti VEGF antibody <VEGF> antibody G6-31 (Liang et al., J Biol Chem. 2006 Jan 13;281(2):951-61; US 2007/0141065) in PBS (Sigma) was added to the wells and incubated for 1 h on a microtiterplate shaker at room temperature. The wells were washed three times with 300µl PBST (0,2% Tween 20) and bound antibody was detected with 100 µL/well 0.1 µg/ml F (ab') <hFcgamma>POD (Immuno research) in 2% BSA 0,1% Tween 20 as detection antibody for 1 h on a microtiterplate shaker at room temperature. Unbound detection antibody was washed away three times with 300 µL/well PBST and the bound detection antibody was detected by addition of 100 µL ABTS/well. Determination of absorbance was performed on a Tecan Fluor Spectrometer at a measurement wavelength of 405 nm (reference wavelength 492 nm).

VEGF binding: Kinetic characterization of VEGF binding at 37°C by surface plasmon resonance (Biacore)

[0145] In order to further corroborate the ELISA findings the binding of <VEGF> antibodies G6-31 or Avastin and <VEGF-Ang-2> TvAb6 or TvAb-2441-bevacizumab-LC06 or TvAb-2441-bevacizumab-LC08 to VEGF was quantitatively analyzed using surface plasmon resonance technology on a Biacore T100 instrument according to the following protocol and analyzed using the T100 software package: Briefly <VEGF> antibodies were captured on a CM5-Chip via binding to a Goat Anti Human IgG (JIR 109-005-098). The capture antibody was immobilized by amino coupling using standard amino coupling as follows: HBS-N buffer served as running buffer, activation was done by mixture of EDC/NHS with the aim for a ligand density of 700 RU. The Capture-Antibody was diluted in coupling buffer NaAc, pH 5.0, c = 2 µg/mL, finally still activated carboxyl groups were blocked by injection of 1 M Ethanolamine. Capturing of Mabs <VEGF> antibodies was done at a flow of 5 µL/min and c(Mabs<VEGF>) = 10 nM, diluted with running buffer + 1 mg/mL BSA; a capture level of approx. 30 RU should be reached. rhVEGF (rhVEGF, R&D-Systems Cat.-No, 293-VE) was used as analyte. The kinetic characterization of VEGF binding to<VEGF> antibodies was performed at 37°C in PBS + 0.005 % (v/v) Tween20 as running buffer. The sample was injected with a flow of 50 µL/min and an association of time 80 sec. and a dissociation time of 1200 sec with a concentration series of rhVEGF from 300 - 0.29 nM. Regeneration of free capture antibody surface was performed with 10 mM Glycin pH 1.5 and a contact time of 60 sec after each analyte cycle. Kinetic constants were calculated by using the usual double referencing method (control reference: binding of rhVEGF to capture molecule Goat Anti Human IgG, blanks on the measuring flow cell, rhVEGF concentration "0", Model: Langmuir binding 1:1, (Rmax set to local because of capture molecule binding). Figure 11 shows a schematic view of the Biacore assay.

ANG-2 binding ELISA

[0146] The binding properties of the tetravalent antibodies (TvAb) was evaluated in an ELISA assay with full-length Angiopoietin-2-His protein (R&D Systems) (Figure 6a). For this sake Falcon polystyrene clear enhanced microtiter plates were coated with 100 µl I µg/mL recombinant human Angiopoietin-2 (R&D Systems, carrier-free) in PBS for 2 h at room temperature or over night at 4°C. The wells were washed three times with 300µl PBST (0,2% Tween 20) and blocked with 200 µl 2% BSA 0,1% Tween 20 for 30 min at room temperature and subsequently washed three times with 300µl PBST. 100 µL/well of a dilution series (40pM-0.01 pM) of purified <VEGF-ANG-2> TvAb and as a reference <ANG-2> antibody Mab536 and VEGF> antibody G6-31 in PBS (Sigma) was added to the wells and incubated for 1 h on a microtiterplate shaker at room temperature. The wells were washed three times with 300µl PBST (0,2% Tween 20) and bound antibody was detected with 100 µL/well 0.1 µg/ml F(ab') <hk>POD (Biozol Cat.No. 206005) in 2% BSA 0,1% Tween 20 as detection antibody for 1 h on a microtiterplate shaker at room temperature. Unbound detection antibody was washed away three times with 300 µL/well PBST and the bound detection antibody was detected by addition of 100 µL ABTS/well. Determination of absorbance was performed on a Tecan Fluor Spectrometer at a measurement wavelength of 405 nm (reference wavelength 492 nm).

Comparative binding to ANG-1 and ANG-2 (ANG-1 and ANG-2 binding ELISA)

[0147] The binding properties of antibodies were evaluated in an ELISA assay with full-length Angiopoietin-2-His protein (R&D Systems #623-AN/CF or in house produced material) or Angiopoietin-1-His (R&D systems #923-AN). Therefore 96 well plates (Falcon polystyrene clear enhanced microtiter plates or Nunc Maxisorb) were coated with 100 µl I µg/mL recombinant human Angiopoietin-1 or Angiopoietin-2 (carrier-free) in PBS (Sigma) for 2 h at room temperature or over night at 4°C. The wells were washed three times with 300µl PBST (0,2% Tween 20) and blocked with 200 µl 2% BSA 0,1% Tween 20 for 30 min at room temperature and subsequently washed three times with 300µl PBST. 100 µL/well of a dilution series (40pM-0.01 pM) of purified test antibody in PBS was added to the wells and incubated for 1 h on a microtiterplate shaker at room temperature. The wells were washed three times with 300µl PBST (0,2% Tween 20) and bound antibody was detected with 100 µL/well 0.1 µg/ml F(ab') <hk>POD (Biozol Cat.No. 206005) in 2% BSA 0,1% Tween 20 as detection antibody for 1 h on a microtiterplate shaker at room temperature. Unbound detection antibody was washed away three times with 300 µL/well PBST and the bound detection antibody was detected by addition of 100 µL ABTS/well. Determination of absorbance was performed on a Tecan Fluor Spectrometer at a measurement wavelength of 405 nm (reference wavelength 492 nm).

ANG-2 binding BIACORE

[0148] Binding of the antibodies to the antigen e.g. human ANG-2 were investigated by surface plasmon resonance using a BIACORE T100 instrument (GE Healthcare Biosciences AB, Uppsala, Sweden). Briefly, for affinity measurements goat<hIgG-Fcgamma> polyclonal antibodies were immobilized on a CM5 chip via amine coupling for presentation of

the antibodies against human ANG-2 (Figure 6B). Binding was measured in HBS buffer (HBS-P (10 mM HEPES, 150 mM NaCl, 0.005% Tween 20, ph 7.4), 25°C. Purified ANG-2-His (R&D systems or in house purified) was added in various concentrations between 6,25 nM and 200 nM in solution. Association was measured by an ANG-2-injection of 3 minutes; dissociation was measured by washing the chip surface with HBS buffer for 3 minutes and a KD value was estimated using a 1:1 Langmuir binding model. Due to heterogenity of the ANG-2 preparation no 1:1 binding could be observed; KD values are thus only relative estimations. Negative control data (e.g. buffer curves) were subtracted from sample curves for correction of system intrinsic baseline drift and for noise signal reduction. Biacore T100 Evaluation Software version 1.1.1 was used for analysis of sensorgrams and for calculation of affinity data. Alternatively, Ang-2 could be captured with a capture level of 2000-1700 RU via a PentaHisAntibody (PentaHis-Ab BSA-free, Qiagen No. 34660) that was immobilized on a CM5 chip via amine coupling (BSA-free) (see below).

Inhibition of huANG-2 binding to Tie-2 (ELISA)

[0149] The interaction ELISA was performed on 384 well microtiter plates (MicroCoat, DE, Cat.No. 464718) at RT. After each incubation step plates were washed 3 times with PBST. ELISA plates were coated with 0.5 µg/ml Tie-2 protein (R&D Systems, UK, Cat.No.313-TI) for at least 2 hours (h).Thereafter the wells were blocked with PBS supplemented with 0.2% Tween-20 and 2% BSA (Roche Diagnostics GmbH, DE) for 1 h. Dilutions of purified antibodies in PBS were incubated together with 0.2 µg/ml huAngiopoietin-2 (R&D Systems, UK, Cat.No. 623-AN) for 1 h at RT. After washing a mixture of 0.5 µg/ml biotinylated anti-Angiopoietin-2 clone BAM0981 (R&D Systems, UK) and 1:3000 diluted streptavidin HRP (Roche Diagnostics GmbH, DE, Cat.No.11089153001) was added for 1 h. Thereafter the plates were washed 6 times with PBST. Plates were developed with freshly prepared ABTS reagent (Roche Diagnostics GmbH, DE, buffer #204 530 001, tablets #11 112 422 001) for 30 minutes at RT. Absorbance was measured at 405 nm.

ANG-2-VEGF bridging ELISA

[0150] The binding properties of the tetravalent antibodies (TvAb) was evaluated in an ELISA assay with immobilized full-length VEGF165-His protein (R&D Systems) and human ANG-2-His protein (R&D Systems) for detection of bound bispecific antibody (Figure 7). Only a bispecific. <VEGF-ANG-2> TvAb is able to o bind simultaneously to VEGF and ANG-2 and thus bridge the two antigens whereas monospecific "standard" IgG1 antibodies should not be capable of simultaneously binding to VEGF and ANG-2 (Figure 7).

[0151] For this sake Falcon polystyrene clear enhanced microtiter plates were coated with 100 µl 2 µg/mL recombinant human VEGF165 (R&D Systems) in PBS for 2 h at room temperature or over night at 4°C. The wells were washed three times with 300µl PBST (0,2% Tween 20) and blocked with 200 µl 2% BSA 0,1% Tween 20 for 30 min at room temperature and subsequently washed three times with 300µl PBST. 100 µL/well of a dilution series (40pM-0.01 pM) of purified <VEGF-ANG-2> TvAb and as a reference <ANG-2> antibody Mab536 and VEGF> antibody G6-31 in PBS (Sigma) was added to the wells and incubated for 1 h on a microtiterplate shaker at room temperature. The wells were washed three times with 300µl PBST (0,2% Tween 20) and bound antibody was detected by addition of 100 µl 0.5 µg/ml human ANG-2-His (R&D Systems) in PBS. The wells were washed three times with 300µl PBST (0,2% Tween 20) and bound ANG-2 was detected with 100 µl 0.5 µg/mL <ANG-2>mIgG1-Biotin antibody (BAM0981, R&D Systems) for 1 h at room temperature. Unbound detection antibody was washed away with three times 300µl PBST (0,2% Tween 20) and bound antibody was detected by addition of 100 µl 1:2000 Streptavidin-POD conjugate (Roche Diagnostics GmbH, Cat. No. 11089153) 1:4 diluted in blocking buffer for 1h at room temperature. Unbound Streptavidin-POD conjugate was washed away with three-six times 300µl PBST (0,2% Tween 20) and bound Strepatavidin-POD conjugate was detected by addition of 100 µL ABTS/well. Determination of absorbance was performed on a Tecan Fluor Spectrometer at a measurement wavelength of 405 nm (reference wavelength 492 nm).

Demonstration of simultaneous binding of bispecific tetravalent antibody <VEGF-Ang-2> TvAb6 to VEGF-A and Ang-2 by Biacore

[0152] In order to further corroborate the data from the bridging ELISA an additional assay was established to confirm simultaneous binding to VEGF and Ang-2 using surface plasmon resonance technology on a Biacore T100 instrument according to the following protocol and analyzed using the T100 software package (T100 Control, Version 2.01, T100 Evaluation, Version 2.01, T100 Kinetics Summary, Version 1.01): Ang-2 was captured with a capture level of 2000-1700 RU in PBS, 0.005 % (v/v) Tween20 running buffer via a PentaHisAntibody (PentaHis-Ab BSA-free, Qiagen No. 34660) that was immobilized on a CM5 chip via amine coupling (BSA-free). HBS-N buffer served as running buffer during coupling, activation was done by mixture of EDC/NHS. The PentaHis-Ab BSA-free Capture-Antibody was diluted in coupling buffer NaAc, pH 4.5, c = 30 µg/mL, finally still activated carboxyl groups were blocked by injection of 1 M Ethanolamine; ligand densities of 5000 and 17000 RU were tested. Ang-2 with a concentration of 500 nM was captured

by the PentaHis-Ab at a flow of 5 µL/min diluted with running buffer + 1 mg/mL BSA. Subsequently, <Ang-2, VEGF> bispecific antibody binding to Ang-2 and to VEGF was demonstrated by incubation with rhVEGF and formation of a sandwich complex. For this sake, bispecific <VEGF-Ang-2> TvAb6 was bound to Ang-2 at a flow of 50 µL/min and a concentration of 100 nM, diluted with running buffer + 1 mg/mL BSA and simultaneous binding was detected by incubation with VEGF (rhVEGF, R&D-Systems Cat.-No, 293-VE) in PBS + 0.005 % (v/v) Tween20 running buffer at a flow of 50 µL/min and a VEGF concentration of 150 nM. Association time 120 sec, dissociation time 1200 sec. Regeneration was done after each cycle at a flow of 50 µL/min with 2 x 10 mM Glycin pH 2.0 and a contact time of 60 sec. Sensorgrams were corrected using the usual double referencing (control reference: binding of bispecific antibody and rhVEGF to capture molecule PentaHisAb). Blanks for each Ab were measured with rhVEGF concentration "0". A scheme of the Biacore assay is shown in Figure 13. An alternative Biacore assay format is shown in Figure 15.

Generation of HEK293-Tic2 cell line

[0153] In order to determine the interference of Angiopoietin-2 antibodies with ANGPT2 stimulated Tie2 phosphorylation and binding of ANGPT2 to Tie2 on cells a recombinant HEK293-Tie cell line was generated. Briefly, a pcDNA3 based plasmid (RB22-pcDNA3 Topo hTie2) coding for full-length human Tie2 (SEQ ID 108) under control of a CMV promoter and a Neomycin resistance marker was transfected using Fugene (Roche Applied Science) as transfection reagent into HEK293 cells (ATCC) and resistant cells were selected in DMEM 10 % FCS, 500µg/ml G418. Individual clones were isolated via a cloning cylinder, and subsequently analyzed for Tie2 expression by FACS. Clone 22 was identified as clone with high and stable Tie2 expression even in the absence of G418 (HEK293-Tie2 clone22). HEK293-Tie2 clone22 was subsequently used for cellular assays: ANGPT2 induced Tie2 phosphorylation and ANGPT2 cellular ligand binding assay.

ANGPT2 induced Tie2 phosphorylation assay

[0154] Inhibition of ANGPT2 induced Tie2 phosphorylation by ANGPT2 antibodies was measured according to the following assay principle. HEK293-Tie2 clone22 was stimulated with ANGPT2 for 5 minutes in the absence or presence of ANGPT2 antibody and P-Tie2 was quantified by a sandwich ELISA. Briefly, 2x105 HEK293-Tie2 clone 22 cells per well were grown over night on a Poly-D-Lysine coated 96 well- microtiter plate in 100µl DMEM, 10% FCS, 500 µg/ml Geneticin. The next day a titration row of ANGPT2 antibodies was prepared in a microtiter plate (4-fold concentrated, 75µl final volume/well, duplicates) and mixed with 75µl of an ANGPT2 (R&D systems # 623-AN] dilution (3.2 µg/ml as 4-fold concentrated solution). Antibodies and ANGPT2 were pre-incubated for 15 min at room temperature. 100 µl of the mix were added to the HEK293-Tie2 clone 22 cells (pre-incubated for 5 min with 1 mM NaV3O4, Sigma #S6508) and incubated for 5 min at 37°C. Subsequently, cells were washed with 200µl ice-cold PBS + 1mM NaV3O4 per well and lysed by addition of 120µl lysis buffer (20 mM Tris, pH 8.0, 137 mM NaCl, 1% NP-40, 10% glycerol, 2mM EDTA, 1 mM NaV3O4, 1 mM PMSF and 10 µg/ml Aprotinin) per well on ice. Cells were lysed for 30 min at 4°C on a microtiter plate shaker and 100 µl lysate were transferred directly into a p-Tie2 ELISA microtiter plate (R&D Systems, R&D #DY990) without previous centrifugation and without total protein determination. P-Tie2 amounts were quantified according to the manufacturer's instructions and IC50 values for inhibition were determined using XLfit4 analysis plug-in for Excel (Dose-response one site, model 205). IC50 values can be compared within on experiment but might vary from experiment to experiment.

VEGF induced HUVEC proliferation assay

[0155] VEGF induced HUVEC (Human Umbilical Vein Endothelial Cells, Promocell #C-12200) proliferation was chosen to measure the cellular function of VEGF antibodies. Briefly, 5000 HUVEC cells (low passage number, ≤ 5 passages) per 96 well were incubated in 100µl starvation medium (EBM-2 Endothelial basal medium 2, Promocell # C-22211, 0.5% FCS, Penicilline/Streptomycine) in a collagen 1-coated BD Biocoat Collagen I 96-well microtiter plate (BD #354407 / 35640 over night. Varying concentrations of antibody were mixed with rhVEGF (30 ngl/ml final concentration, BD # 354107) and pre-incubated for 15 minutes at room temperature. Subsequently, the mix was added to the HUVEC cells and they were incubated for 72 h at 37°C, 5% CO2. On the day of analysis the plate was equilibrated to room temperature for 30 min and cell viability/proliferation was determined using the CellTiter-GloTM Luminescent Cell Viability Assay kit according to the manual (Promega, # G7571/2/3). Luminescence was determined in a spectrophotometer.

Design of tetravalent bispecific and tetravalent monospecific antibodies (reference)

[0156] The bispecific antibodies binding to VEGF (VEGF-A) and ANG-2 (Angiopoietin-2) comprise a first antigen-binding site that binds to VEGF and a second antigen-binding site that binds to ANG-2. As first antigen-binding site

binding to VEGF, e.g. the heavy chain variable domain of SEQ ID NO: 23, and the light chain variable domains of SEQ ID NO: 24 which are both derived from the human phage display derived anti-VEGF antibody G6-31 which is described in detail in Liang, W.C., et al., J Biol Chem. 281(2) (2006) 951-61 and in US 2007/0141065, can be used. Alternatively e.g. the second antigen-binding site specifically binding to VEGF comprises the heavy chain variable domains of SEQ ID NO: 7, or SEQ ID NO: 100, and the light chain variable domains SEQ ID NO:8 or SEQ ID NO: 101 from the anti-VEGF antibodies <VEGF>bevacizumab and <VEGF>B20-4.1., preferably from <VEGF>bevacizumab.

[0157] As second antigen-binding site comprises the heavy chain variable domains SEQ ID NO: 31, and the light chain variable domains SEQ ID NO: 32 or SEQ ID NO: 32 with the mutations T92L, H93Q andW94T (Kabat numbering), which are both derived from the human anti-ANG-2 antibody <ANG-2> Mab536 which is described in detail in Oliner, J., et al., Cancer Cell. 6(5) (2004) 507-16, and in US 2006/0122370, can be used. Alternatively e.g. the second antigen-binding site specifically binding to ANG-2 comprises the heavy chain variable domains of SEQ ID NO: 44, SEQ ID NO: 52, SEQ ID NO: 60, SEQ ID NO: 68, SEQ ID NO: 76, SEQ ID NO: 84 or SEQ ID NO: 92, and the light chain variable domains SEQ ID NO: 45, SEQ ID NO: 53, SEQ ID NO: 61, SEQ ID NO: 69, SEQ ID NO: 77, SEQ ID NO: 85, SEQ ID NO: 93 from the anti-ANG-2 antibodies <ANG-2> Ang2s_R3_LC03, <ANG-2>Ang2i_LC06, <ANG-2>Ang2i_LC07, <ANG-2> Ang2k_LC08, <ANG-2> Ang2s_LC09, <ANG-2> Ang2i_LC10, or <ANG-2> Ang2k_LC11, preferably from <ANG-2>Ang2i_LC06, or <ANG-2> Ang2k_LC08.

[0158] To generate agents that combine features of both antibodies, novel tetravalent bispecific antibody-derived protein entities were constructed. In these molecules, recombinant single-chain binding molecules of one antibody are connected via recombinant protein fusion technologies to the other antibody which was retained in the format of a full-length IgG1. This second antibody carries the desired second binding specificity.

[0159] By gene synthesis and recombinant molecular biology techniques, the heavy chain variable domain (VH) and the light chain variable domain (VL) of the respective antibody were linked by a glycine serine (G4S)3 or (G4S)4 single-chain-linker to give a single chain Fv (scFv), which was attached to the C- terminus of the other antibody heavy chain using a (G)6- or (G4S)3-linker.

[0160] In addition, cysteine residues were introduced in the VH (including Kabat position 44,) and VL (including Kabat position 100) domain of the scFv binding to ANG-2 or VEGF as described earlier (e.g. WO 94/029350; Reiter, Y., et al., Nature biotechnology (1996) 1239-1245; Young, N.M., et al, FEBS Letters (1995) 135-139; or Rajagopal, V., et al., Protein Engineering (1997) 1453-59).

[0161] All these molecules were recombinantly produced, purified and characterized and protein expression, stability and biological activity was evaluated.

[0162] A summary of the bispecific antibody designs that were applied to generate tetravalent bispecific <VEGF-ANG-2>, <ANG-2-VEGF> antibodies and tetravalent monospecific <ANG-2> antibodies is given in Table 3. For this study, we use the term 'TvAb' to describe the various tetravalent protein entities.

[0163] In order to obtain the bispecific tetravalent antibodies <VEGF-ANG-2> TvAb5 and TvAb6 the single chain Fv (scFv) binding to Angiopoietin-2 derived from the heavy chain variable domain (VH) of SEQ ID NO: 31, and the light chain variable domain (VL) of SEQ ID NO: 32 with the mutations T92L, H93Q andW94T derived from the human anti-ANG-2 antibody <ANG-2> Mab536 was fused to the sequence corresponding to the C-terminus of the heavy chain vector of the human anti-VEGF antibody <VEGF> G6-31 of SEQ ID NO: 23 and co-expressed with the respective light chain expression vector based on SEQ ID NO: 24. A representation of the designed formats is shown in Figure 1B and listed in Table 3.

[0164] In order to obtain the bispecific tetravalent antibodies TvAb9 and TvAb15 the single chain Fv (scFv) binding to VEGF derived from the heavy chain variable domain (VH) of SEQ ID NO: 23, and the light chain variable domain (VL) of SEQ ID NO: 24 derived from the human anti-VEGF antibody <VEGF> G6-31 was fused to the sequence corresponding to the C-terminus of the heavy chain vector of the human anti-ANG-2 antibody <ANG-2> Mab536 of SEQ ID NO: 31 and co-expressed with the respective light chain expression vector based on SEQ ID NO: 32. A representation of the designed formats is shown in Figure 1B and listed in Table 3.

Table 3 - The different bispecific tetravalent antibody formats with C- terminal scFv attachments and the corresponding TvAb-nomenclature. An "-" in the table means "not present"

Molecule Name (TvAbnomenclature for bispecific antibodies)	Antibody backbone derived from	scFv derived from	Variable Domains VH and VL: SEQ ID NO :	Position of scfv at tached to antibody	Single-chain-linker	Peptide-linker	scFv disulfide VH44/ VL100 stabilized
G6=31 (1000)	<hVEGF> G6-31	-	23 + 24	-	-	-	-

(continued)

Molecule Name (TvAbnomenclature for bispecific antibodies)	Antibody backbone derived from	scFv derived from	Variable Domains VH and VL: SEQ ID NO :	Position of scfv at tached to antibody	Single-chain-linker	Peptide-linker	scFv disulfide VH44/ VL100 stabilized
Mab536 (1000)	<ANG-2> Mab536	-	31 + 32	-	-	-	-
bevacizumab	<VEGF> bevacizu mab	-	23 + 24	-	-	-	-
Ang2i_LC06 (LC06)	<ANG-2>Ang2i_ LC06	-	52 + 53	-	-	-	-
Ang2k_LC06 (LC08)	<ANG-2>Ang2k LC08	-	68 + 69				
TvAb5 (2310)	<VEGF> G6-31	<ANG-2> Mab53 6	23+24, 31 + 32 with the mutations T92L, H93Q and W 94T	C-term. HC	(G4S)3	(G)6	-
TvAb6 (2331)	<VEGF> G6-31	<ANG-2> Mab53 6	23+24, 31 + 32 with the mutations T92L, H93Q andW94T	C-term. HC	(G4S)3	(G4S)3	scFv disulfide VH44/VL 100 stabilized
TvAb9 (2330)	<ANG-2> Mab536	<VEGF > G6-31	31 + 32, and 23+24	C-term. HC	(G4S)3	(G4S)3	-
TvAb15 (2431)	<ANG-2> Mab536	<VEGF 31 + 32, > G6-31	31 + 32, and 23+24	C-term. HC	(G4S)4	(G4S)3	scFv disulfide VH44NL 100 stabilized
TvAb-2441-bevacizumab -LC06	bevacizu mab	LC06	7 + 8 and 52 + 53	C-term. HC	(G4S)4	(G4S)4	scFv disulfide VH44NL 100 stabilized
TvAb-2441-bevacizumab -LC08	bevacizu mab	LC08	7 + 8 and 68 + 69	C-term. HC	(G4S)4	(G4S)4	scFv disulfide VH44NL 100 stabilized

(continued)

Molecule Name (TvAbnomenclature for bispecific antibodies)	Antibody backbone derived from	scFv derived from	Variable Domains VH and VL: SEQ ID NO :	Position of scfv at tached to antibody	Single-chain-linker	Peptide-linker	scFv disulfide VH44/ VL100 stabilized
TvAb-3421_bevacizumab_LC0 6	bevacizumab	LC06	+ 8 and 52 + 53	N-term. HC	(G4S)4	(G4S)2	scFv disulfide VH44NL 100 stabilized
TvAb-4421_bevacizumab _LC06	bevacizumab	LC06	7 + 8 and 52 + 53	C-term LC	(G4S)4	(G4S)2	scFv disulfide VH44NL 100 stabilized
TvAb-4461_bevacizumab _LC06	bevacizumab	LC06	+ 8 and 52 + 53	C-term LC	(G4S)4	(G4S)6	scFv disulfide VH44/VL 100 stabilized

[0165] The TvAb formats are based e.g. on

a) aa) the human anti-VEGF antibody <VEGF> G6-31 and ab) two single chain Fv (scFv) binding to Angiopoietin-2 derived from the heavy chain variable domain (VH) of SEQ ID NO: 31, and the light chain variable domain (VL) of SEQ ID NO: 32 with the mutations T92L, H93Q andW94T, which are linked to the C-terminus of the heavy chain of the anti-VEGF antibody <VEGF> G6-31 (SEQ ID NO: 23); or

b) ba) the human anti-ANG-2 antibody <ANG-2> Mab536 and bb) two single chain Fv (scFv) binding to VEGF derived from the heavy chain variable domain (VH) of SEQ ID NO: 23, and the light chain variable domain (VL) of SEQ ID NO: 24, which are linked to the C-terminus of the heavy chain of the anti-ANG-2 antibody <ANG-2> Mab536 (SEQ ID NO: 31);or

c) ca) the human anti-VEGF antibody <VEGF> bevacizumab (Avastin) and cb) two single chain Fv (scFv) binding to Angiopoietin-2 derived from the heavy chain variable domain (VH) of SEQ ID NO: 52 or of SEQ ID NO: 68, and the light chain variable domain (VL) of SEQ ID NO: 53 or of SEQ ID NO: 69, which are linked to the C-terminus of the heavy chain of the anti-VEGF antibody <VEGF> bevacizumab (Avastin) (the Sequences of the resulting fusion peptide are SEQ ID NO: 102 or SEQ ID NO: 103, which are co-expressed with the light chain of bevacizumab SEQ ID NO: 104. (Alternatively two single chain Fv (scFv) binding to Angiopoietin-2 can also be linked to the C-terminus of the light chain or the N-terminus of the heavy chain).

[0166] Alternativly to the single two single chain Fv (scFv) also single chain Fab fragments can be used as described above (using peptide connectors for the fusion to C or N-termini), in EP Appl. No 09004909.9 and in Example 10.

Example 1 (reference)

Expression & purification of bispecific tetravalent antibodies

<VEGF-ANG-2> TvAb5, TvAb6, TvAb-2441-bevacizumab-LC06 and TvAb-2441-bevacizumab -LC08

[0167] Light and heavy chains of the corresponding tetravalent bispecific antibodies TvAb5 and TvAb6 were constructed in genomic expression vectors as described above). The plasmids were amplified in E. coli, purified, and subsequently transfected for transient expression of recombinant proteins in HEK293-F cells (utilizing Invitrogen's FreeStyle 293 system). After 7 days, HEK 293-F cell supernatants were harvested, filtered and the bispecific antibodies were purified

by protein A and size exclusion chromatography. Homogeneity of all bispecific antibody constructs was confirmed by SDS-PAGE under non reducing and reducing conditions and analytical size exclusion chromatography. Under reducing conditions (Figure 3), polypeptide heavy chains of <VEGF-ANG-2> TvAb6 carrying the C-terminal scFv fusion showed upon SDS-PAGE apparent molecular sizes of ca. 75 kDa analogous to the calculated molecular weights. Mass spectrometry confirmed the identity of the purified antibody constructs. Expression levels of all constructs were analysed by Protein A HPLC and were similar to expression yields of 'standard' IgGs. Protein yields achieved up to 150 mg of TvAb6 <VEGF-ANG-2> per liter of cell-culture supernatant as determined by Protein A HPLC.

[0168] Size exclusion chromatography analysis of the purified non-disulfide stabilized construct TvAb5 with C-terminal fused scFv at the heavy chain showed compared to 'standard' IgGs an increased tendency to aggregate again after purification of monomeric antibody via size exclusion chromatography (so-called "daisy chain" phenomenon). This finding has been supported by other examples (Rajagopal, V., et al., Prot. Engin. (1997) 1453-1459; Kobayashi, H., et al, Nucl Med Biol. (1998) 387-393 or Schmidt, M., et al, Oncogene (1999) 18, 1711 - 1721) showing that molecules that contained scFvs that were not stabilized by interchain disulfides between VH and VL exhibited an increased tendency to aggregate and reduced yields. To address the problems with aggregation of such bispecific antibodies, disulfide-stabilization of the scFv moieties was applied. For that we introduced single cysteine replacements within VH and VL of the scFv at defined positions (positions VH44/VL100 according to the Kabat numbering scheme). These mutations enable the formation of stable interchain disulfides between VH and VL, which in turn stabilize the resulting disulfide-stabilized scFv module. Introduction of the VH44/VL100 disulfides in the scFv at the C-terminus of the Fv in TvAb6 <VEGF-ANG-2> lead to a stable tetravalent antibody that showed no aggregation tendency any longer after purification and remained in a monomeric state (Figure 4). In addition, TvAb6 <VEGF-ANG-2> showed no increase in aggregation tendency upon repeated freeze-thaw cycles e.g. at the concentration applied for in vitro and in vivo of 3 mg/kg.

[0169] All other TvAb molecules described in Table 3 (e.g. TvAb-2441-bevacizumab - LC06 and TvAb-2441-bevacizumab -LC08) were prepared and analytically characterized analogously to the procedure described.

Example 2 (reference)

Simultaneous binding of bispecific tetravalent antibody <VEGF-ANG-2> TvAb6, TvAb-2441-bevacizumab -LC06 and TvAb-2441-bevacizumab -LC08 to VEGF-A and ANG-2

[0170] The binding of the scFv modules and of the Fvs retained in the IgG-module of the different bispecific antibody formats were compared to the binding of the 'wildtype' IgGs from which the binding modules and bispecific antibodies were derived. These analyses were carried out at equimolar concentrations by performing biochemical binding ELISAs and by applying Surface Plasmon Resonance (Biacore).

[0171] For <VEGF-ANG-2> TvAb6 is was shown by VEGF binding ELISA as described above that it binds to VEGF comparable to its parent antibody G6-31 at an equimolar concentration of 0.625 pM (Figure 5). This finding could be expected as the Fv region of the TvAb is identical to that of G6-31. The slight difference between <VEGF-ANG-2> TvAb6 and <VEGF> G6-31 is due to small differences in protein concentration and a slight steric interference of the C-terminal scFv with binding of the <hFc>-POD detection antibody and can be overcome by application of a <hk> POD (Biozol Cat.No. 206005) detection antibody like used for the ANG-2 binding ELISA.

[0172] Using Biacore these findings were confirmed using a classical concentration series at 37 °C (Figure 11). These data showed fast Kon-rates k(a) of 4.7-4.8 E+6 1/(Ms), saturation was reached with the highest concentrations of VEGF. Koff-rates reached limits of technical specification.(i.e. 5 x E-6 (s/s) probably due to still bivalent binding (avidity effect) under this conditions as a consequence of the dimeric analyte rhVEGF, although a very low ligand density was used resulting in final VEGF-response of 10-15 RU. Nevertheless, the kinetic constants of the different <VEGF> antibodies could be compared by this method and within the error of the method there was no significant difference in the kinetic constants of the tetravalent bispecific antibody <VEGF-Ang-2> TvAb6 and the original antibody <VEGF> G6-31 detectable. The kinetic constants for <VEGF-Ang-2> TvAb6 and <VEGF> G6-31 under these conditions were virtually identical by this method. Thus, it can be concluded that TvAb6 completely retained its VEGF binding properties. Tab. 4 shows the respective kinetic constants and Figure 12 shows the kinetic characteristics of the two <VEGF> antibodies <VEGF-Ang-2> TvAb6 and <VEGF> G6-31 in a Ka-Kd plot.

Tab. 14: Kinetic properties of <VEGF-Ang-2> TvAb6 and <VEGF> G6-31

Measured at 37°C	**Ka**	**kd**	**t1/2**	**KD**
Antibodies	[1/(Ms)]	[1/s]	[min]	[M]
<VEGF> G6-31	4.83E+06	9.33E-06	1237.8	1.93E-12
<VEGF-Ang-2> TvAb6	4.72E+06	7.24E-06	1596.7	1.53E-12

[0173] In a further experiment it was shown by ANG-2 binding ELISA using a <hk>-POD detection antibody (Biozol Catalogue No. 206005) as described above that <VEGF-ANG-2> TvAb6 binds to ANG-2 in a manner comparable to that of Mab536 at an equimolar concentration of 0.039 pM (Figure 6A). This showed that the scFv module of TvAb6 retained its binding properties in the TvAb construct.

[0174] In order to further corroborate this finding <ANG-2> Mab536 and <VEGF-ANG-2> TvAb6 were immobilized by a secondary antibody on a Biacore CM5 chip and binding kinetics to human ANG-2 were determined. Due to heterogeneity of the ANG-2 preparation no 1:1 binding can be observed; KD values are thus only relative estimations. The Biacore analysis showed that <VEGF-ANG-2> TvAb6 has an estimated KD value of 4.4 nM for ANG-2. In comparison, Mab536 has an estimated KD value of 1.6 nM. Within the error of the method no difference in binding mode and affinities between <ANG-2> Mab536 and <VEGF-ANG-2> TvAb6 could be observed (Figure 6B). Thus, it can be concluded that the scFv module of TvAb6 completely retained its binding properties in the TvAb construct.

[0175] In order to prove that <VEGF-ANG-2> TvAb6 was able to bind simultaneously to VEGF and ANG-2 bridging ELISA assays and Biacore assays as described above were applied.

[0176] By applying the VEGF-ANG-2-bridging ELISA described above it was shown that only <VEGF-ANG-2> TvAb6 was able to bind simultaneously to VEGF and ANG-2 at an equimolar concentration of 0.625 pM whereas the monospecific "standard" IgG1 antibodies <ANG-2> Mab536 and <VEGF> G6-31 were not capable of simultaneously binding to VEGF and ANG-2 (Figure 7).

[0177] Figure 14 shows the respective data from the Biacore assay. Simultaneous binding of both antigens Ang-2 and VEGF could be shown for the tetravalent bispecific antibody <VEGF-Ang-2> TvAb6. Negative controls were as expected: The monospecific antibody <Ang-2> Mab536 showed only binding to Ang-2, but no VEGF-binding. The monospecific antibody <VEGF> G6-31 showed binding to VEGF but no binding to Ang-2 at all (data not shown). From the relative response units of the tetravalent bispecific antibody <VEGF-Ang-2> TvAb6 binding to the Ang-2 coated surface, and subsequent binding to dimeric VEGF binding the stochiometry could be calculated to be in the range from 1:1 to 1:1.4. Taken together, by applying the described ELISA and Biacore assays it was shown that only <VEGF-Ang-2> TvAb6 was able to bind simultaneously to VEGF and Ang-2 whereas the monospecific "standard" IgG1 antibodies <Ang-2> Mab536 and <VEGFY G6-31 were not capable of simultaneously binding to VEGF and Ang-2 (Figure 15).

[0178] Similar results were obtained with the constructs TvAb-2441-bevacizumab-LC06 and TvAb-2441-bevacizumab-LC08 in an analogous Biacore assay shown in Fig. 15A. Binding of the antibodies to the antigen e.g. human ANG-2 and VEGF were investigated by surface plasmon resonance using a BIACORE T100 instrument (GE Healthcare Biosciences AB, Uppsala, Sweden). Briefly, for affinity measurements goat<hIgG-Fc□> polyclonal antibodies were immobilized on a CM4 chip via amine coupling for presentation of the bispecific antibodies against human ANG-2 and VEGF. Binding was measured in HBS buffer (HBS-P (10 mM HEPES, 150 mM NaCl, 0.005% Tween 20, ph 7.4), 25°C. Purified ANG-2-His (R&D systems or in house purified) was added in various concentrations between 6,25 nM and 200 nM in solution. Association was measured by an ANG-2-injection of 3 minutes; dissociation was measured by washing the chip surface with HBS buffer for 3 minutes and a KD value was estimated using a 1:1 Langmuir binding model. Due to heterogenity of the ANG-2 preparation no 1:1 binding could be observed; KD values are thus only relative estimations.

[0179] VEGF (R&D systems) was added in various concentrations between 6,25 nM and 200 nM in solution. Association was measured by an VEGF-injection of 3 minutes; dissociation was measured by washing the chip surface with HBS buffer for 3 minutes and a KD value was estimated using a 1:1 Langmuir binding model.

[0180] The order of injection of the binding partners can switched, first VEGF and then Ang2 or vice versa.

[0181] Negative control data (e.g. buffer curves) were subtracted from sample curves for correction of system intrinsic baseline drift and for noise signal reduction. Biacore T100 Evaluation Software version 1.1.1 was used for analysis of sensorgrams and for calculation of affinity data.

Antibody	Affinity hAng-2	Affinity hVEGF
TvAb-2441-bevacizumab-LC06	2,3 nM	0,35 nM
TvAb-2441-bevacizumab-LC08	0,7 nM	0,34 nM
G6-31	--	< 0,1 nM
MAb536	3 nM	--
bevacizumab	--	0,59 nM

[0182] Finally, simultaneous binding of TvAb-2441-bevacizumab-LC06 and TvAb-2441-hevacizumab-LC08 could be shown by incubating with ANGPT2 and VEGF in a consecutive manner. As shown in Figure 15B ANGPT2 and VEGF

can bind simultaneously to the bispecific antibodies.

Example 3 (reference)

In vivo efficacy of disulfide-stabilized bispecific tetravalent antibody <VEGF-ANG-2> TvAb6 in comparison to <ANG-2> Mab536, <VEGF> G6-31 and the combination of Mab536 and G6-31 in the staged subcutaneous Colo205 xenograft model in Scid beige mice

[0183] The purified disulfide-stabilized <VEGF-ANG-2> TvAb6 (n00.2331 see Table 3) was compared to the antibodies <ANG-2> Mab536, <VEGF> G6-31 and the combination of <ANG-2> Mab536 and <VEGF> G6-31 in two staged subcutaneous Colo205 xenograft model studies (Ang2_PZ_Colo205_003 and Ang2_PZ_Colo205_005) in female Scid beige mice at different doses.

[0184] Antibodies: <ANG-2> Mab536 was provided as frozen stock solution (c = 4.5 mg/mL), <VEGF> G6-31 was provided as frozen solution (c = 0.6 mg/mL) and <VEGF-ANG-2> TvAb6 was provided as frozen stock solution (c = 0.5 mg/mL) in 20 mM Histidine, 140 mM NaCl, pH 6.0. Antibody solution was diluted appropriately in PBS from stock prior injections where required and PBS was applied as vehicle.

[0185] Cell lines and culture conditions: Colo205 human colorectal cancer cells were originally obtained from ATCC and after expansion deposited in the Roche Penzberg internal cell bank. Tumor cell line was routinely cultured in RPMI 1640 medium (PAA, Laboratories, Austria) supplemented with 10 % fetal bovine serum (PAA Laboratories, Austria) and 2 mM L-glutamine, at 37 °C in a water-saturated atmosphere at 5 % CO_2. Passage 2-5 was used for transplantation.

[0186] Animals: Female SCID beige mice; age 4-5 weeks at arrival (purchased from Charles River Germany) were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government. After arrival animals were maintained in the quarantine part of the animal facility for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on regular basis. Diet food (Provimi Kliba 3337) and water (acidified pH 2.5-3) were provided ad libitum. Age of mice at start of the study was about 10 weeks. Monitoring: Animals were controlled daily for clinical symptoms and detection of adverse effects. For monitoring throughout the experiment body weight of animals was documented and tumor volume was measured by caliper after staging.

[0187] Tumor cell injection: At day of injection Colo205 cells were centrifuged, washed once and resuspended in PBS. After an additional washing with PBS cell concentration and cell size were determined using a cell counter and analyzer system (Vi-CELL, Beckman Coulter). For injection of Colo205 cells, the final titer was adjusted to 5.0 x 10E7 cells/ml, viability ca. 90%. Subsequently 100 µl of this suspension corresponding to 2.5*106 cells per animal was injected s.c. into the right flank of the mice.

[0188] Treatment of animals started at day of randomisation, 16 days after cell transplantation (study Ang2_PZ_Colo205_003) and 14 days after cell transplantation (study Ang2_PZ_Colo205_005) at a mean tumor volume of 100 mm3 or 150 mm3, respectively.

[0189] Dose schedule until Day 74 (see Fig 8A) of Study Ang2_PZ_Colo205_003:

Group	No of animals	Compound	Dose (mg/kg)	Route/Mode of administration	No of treatments	Cumulative Dosis mg/kg
1	10	Vehicle		i.p. once weekly	4	
2	10	<VEGF> G6-31	6 mg/kg	i.p. once weekly	8	48
3	10	<ANG-2> Mab536	6 mg/kg	i.p. once weekly	8	48
4	10	<VEGF> G6-31 +	5mg/kg +	i.p. once weekly	8	40
		<ANG-2> Mab536	6 mg/kg	i.p. once weekly	8	48
5	10	<VEGF-ANG-2> TvAb6	7mg/kg	i.p. once weekly	8	56

[0190] In the study Ang2_PZ_Colo205_003 <VEGF-ANG-2> TvAb6 was by mistake underdosed with respect to an

equimolar ratio. The dose of <VEGF-ANG-2> TvAb6 was adjusted in study Ang2_PZ_Colo205_005 so that the animals received an equimolar ratio of ANG-2 and VEGF binding sites by <VEGF-ANG-2> TvAb6 as well as the combination of <VEGF> G6-31 and <ANG-2> Mab536.

[0191] Tumor growth inhibition until Day 74 (see Fig 8a) Ang2-PZ-Colo205_003 study:

<VEGF-ANG-2> TvAb6 at a dose of 7 mg/kg exhibited efficacy comparable to that of the combination of <VEGF> G6-31 at 5 mg/kg and <ANG-2> Mab536 at 6 mg/kg and <VEGF> G6-31 as single agent at a dose of 6 mg/kg (Figure 8A) and was superior to single agent <ANG-2> Mab536 at a dose of 6 mg/kg. As the subcutaneous Colo205 model is very responsive to the <VEGF> G6-31 antibody that blocks human as well as murine VEGF resulting in almost complete tumor growth inhibition <VEGF-ANG-2> TvAb6 could thus not be differentiated from G6-31 as single agent (6 mg/kg) under the chosen experimental conditions, while <VEGF-ANG-2> TvAb6 showed a comparable inhibition like the combination of <ANG-2> Mab536 and <VEGF> G6-31 at a clearly lower cumulative dose (<VEGF-ANG-2> TvAb6 =56 mg/kg antibody compared to the combination of <ANG-2> Mab536 and <VEGF> G6-31 = 40 + 48 = 88 mg/kg antibody).

[0192] Dose schedule of Study until Day 63 Ang2_PZ_Colo205_005:

Group	No of animals	Compound	Dose (mg/kg)	Route/ Mode of administration	No of treatment	Cumu -lative Dosis
6	10	Vehicle		i.p. once weekly	6	
7	10	<VEGF> G6-31	3	i.p. once weekly	7	21 mg/ kg
8	10	<VEGF> G6-31 + <ANG-2> Mab536	3 3	i.p. once weekly i.p. once weekiyl	7 7	21 mg/kg 21 mg/kg
9	10	<ANG-2> Mab536	3	i.p. once weekly	7	21 mg/kg
10	10	<VEGF-ANG-2> TvAb6	4	i.p. once weekly	7	28 mg/kg

[0193] Tumor growth inhibition until day 63 Ang2_PZ_Colo205_005 study:

<VEGF-ANG-2> TvAb6 at a dose of 4 mg/kg exhibited efficacy comparable to that of the combination of <VEGF> G6-31 and <ANG-2> Mab536 at 3 mg/kg each and was superior to either single agent <VEGF> G6-31 as well as <ANG-2> Mab536 at a dose of 3 mg/kg (Figure 8B). This is the first example showing that at a lower dose (with respect to the summarized concentration of antibody - the cumulative dose of the combination is 21+21 = 42 m/kg versus 28 mg/kg of the bispecific antibody TvAb6) a bispecific antibody targeting VEGF and ANG-2 can result in strong anti-tumor efficacy comparable to the combination of the respective single agents blocking VEGF and ANG-2 and superior to either single agent.

Example 4 (reference)

Blocking of VEGF-induced tube formation

[0194] In order to confirm that the anti-VEGF related activities were retained in the bispecific tetravalent <VEGF-ANG-2> TvAb6 is was shown in a VEGF induced tube formation assay AngioKit TCS CellWorks (CellSystems) that <VEGF-ANG-2> TvAb6 mediated dose dependent inhibition of tube formation comparable to the monospecific antibody <VEGF> G6-31. The AngioKit TCS CellWorks assay was performed according to the following procedure: Cells were stimulated each time with 2 ng/ml VEGF before treatment with antibodies on day 1, 4, 7 and 9. Vascular tubes were visualized by staining of endothelial cells using a CD31-PE antibody (BD Pharmingen #555446) on day 11. Pictures were taken at a magnification of 4x and values for tube length and number of branch points were quantitatively analysed using the Angiogenesis Tube Formation Application Module in MetaMorph (Molecular Devices). Values and standard deviation were calculated by duplicates and analysis of 4 pictures per specimen. Figure 9 shows the respective results and Figure

10 A and B the quantitative analysis. Angiopietin-2 has no influence on tube formation and thus inhibition of ANG-2 was not studied in this assay. The data show that the bispecific <VEGF-ANG-2> TvAb6 and the monospecific <VEGF> G6-31 antibodies are equally efficacious in inhibiting VEGF stimulated tube formation.

Example 5 (reference)

Tie2 phosphorylation

[0195] In order to confirm that the anti-ANGPT2 related activities were retained in the bispecific tetravalent <VEGF-ANGPT2> antibodies TvAb-2441-bevacizumab-LC06 and TvAb-2441-bevacizumab-LC08 it was shown that TvAb-2441-bevacizumab-LC06 and TvAb-2441-bevacizumab-LC08 interfere with ANGPT2 stimulated Tie2 phosphorylation in a comparable manner as their mother clones LC06 and LC08 in the ANGPT2 stimulated Tie2 phosphorylation assay as described above.

[0196] In a first experiment both bispecific antibodies TvAb-2441-bevacizumab-LC06 and TvAb-2441-bevacizumab-LC08 showed a dose-dependent interference with ANGPT2 stimulated Tie2 phosphorylation with IC50 values comparable to those of the mother clones LC06 and LC08 as shown in Figure 16A. TvAb-2441-bevacizumab-LC06 interfered with ANGPT2 stimulated Tie2 phosphorylation with a IC50 value of approx. 721 ng/ml, whereas LC06 interfered with ANGPT2 stimulated Tie2 phosphorylation with a IC50 value of approx. 508 ng/ml. TvAb-2441-bevacizumab-LC08 interfered with ANGPT2 stimulated Tie2 phosphorylation with a IC50 value of approx.364 ng/ml, whereas LC08 interfered with ANGPT2 stimulated Tie2 phosphorylation with a IC50 value of approx.499 ng/ml.

[0197] In a second experiment both bispecific antibodies TvAb-2441-bevacizumab-LC06 and TvAb-2441-bevacizumab-LC08 showed a dose-dependent interference with ANGPT2 stimulated Tie2 phosphorylation with IC50 values comparable to those of the mother clones LC06 and LC08 as shown in Figure 16B. TvAb-2441-bevacizumab-LC06 interfered with ANGPT2 stimulated Tie2 phosphorylation with a IC50 value of approx. 488 ng/ml, whereas LC06 interfered with ANGPT2 stimulated Tie2 phosphorylation with a IC50 value of approx. 424 ng/ml. TvAb-2441-bevacizumab-LC08 interfered with ANGPT2 stimulated Tie2 phosphorylation with a IC50 value of approx. 490 ng/ml, whereas LC08 interfered with ANGPT2 stimulated Tie2 phosphorylation with a IC50 value of approx. 399 ng/ml.

[0198] Taken together these data show that the bispecific tetravalent <VEGF-ANGPT2> antibodies TvAb-2441-bevacizumab-LC06 and TvAb-2441-bevacizumab-LC08 interfere with ANGPT2 stimulated Tie2 phosphorylation in a manner comparable to their mother clones LC06 and LC08 within the error of this cellular assay.

Example 6 (reference)

Inhibition of huANG-2 binding to Tie-2 (ELISA)

[0199] The interaction ELISA was performed on 384 well microtiter plates (MicroCoat, DE, Cat.No. 464718) at RT. After each incubation step plates were washed 3 times with PBST. ELISA plates were coated with 0.5 µg/ml Tie-2 protein (R&D Systems, UK, Cat.No.313-TI) for at least 2 hours (h).Thereafter the wells were blocked with PBS supplemented with 0.2% Tween-20 and 2% BSA (Roche Diagnostics GmbH, DE) for 1 h. Dilutions of purified antibodies in PBS were incubated together with 0.2 µg/ml huAngiopoietin-2 (R&D Systems, UK, Cat.No. 623-AN) for 1 h at RT. After washing a mixture of 0.5 µg/ml biotinylated anti-Angiopoietin-2 clone BAM0981 (R&D Systems, UK) and 1:3000 diluted streptavidin HRP (Roche Diagnostics GmbH, DE, Cat.No.11089153001) was added for 1 h. Thereafter the plates were washed 6 times with PBST. Plates were developed with freshly prepared ABTS reagent (Roche Diagnostics GmbH, DE, buffer #204 530 001, tablets #11 112 422 001) for 30 minutes at RT. Absorbance was measured at 405 nm.

Summary data for Ang2 interaction ELISA:

[0200]

<VEGF-ANG-2> bispecific antibody (or monospecific parent antibodies)	AVG IC50 (ng/ml)	STDEV
	hANG2	
<VEGF-ANG-2>G6_31	>20000	
TvAb-2441_G6_31_Ang2i_LC06	75	39
TvAb-2441_G6_31_Ang2k_LC08	66	31
TvAb-2441_bevacizumab_LC06	44	8

(continued)

<VEGF-ANG-2> bispecific antibody (or monospecific parent antibodies)	AVG IC50 (ng/ml)	STDEV
	hANG2	
TvAb-2441_bevacizumab_LC08	42	11
<ANG-2>Mab 536	15	8
<VEGF>Bevacizumab	>20000	
TvAb-3421_bevacizumab_LC06	31	1
TvAb-4421_bevacizumab_LC06	35	17
TvAb-4461_bevacizumab_LC06	46	10

Example 7 (reference)

Inhibition of hVEGF binding to hVEGF Receptor (ELISA)

[0201] The test was performed on 384 well microtiter plates (MicroCoat, DE, Cat.No. 464718) at RT. After each incubation step plates were washed 3 times with PBST. At the beginning, plates were coated with 0.5 µg/ml hVEGF-R protein (R&D Systems, UK, Cat.No.321-FL) for at least 2 hours (h). Thereafter the wells were blocked with PBS supplemented with 0.2% Tween-20 and 2% BSA (Roche Diagnostics GmbH, DE) for 1 h. Dilutions of purified antibodies in PBS were incubated together with 0.15 µg/ml huVEGF121 (R&D Systems, UK, Cat.No. 298-VS) for 1 h at RT. After washing a mixture of 0.5 µg/ml anti VEGF clone Mab923 (R&D Systems, UK) and 1:2000 horse radish peroxidase (HRP)-conjugated F(ab')2 anti mouse IgG (GE Healthcare, UK, Cat.No.NA9310V) was added for 1 h. Thereafter the plates were washed 6 times with PBST. Plates were developed with freshly prepared ABTS reagent (Roche Diagnostics GmbH, DE, buffer #204 530 001, tablets #11 112 422 001) for 30 minutes at RT. Absorbance was measured at 405 nm.

Summary data for VEGF interaction ELISA:

[0202]

<VEGF-ANG-2> bispecific antibody	AVG IC50 (ng/ml)	STDEV
	VEGF	
<VEGF-ANG-2> G6_31	1431	130
TvAb-2441_G6_31_Ang2i_LC06	1654	213
TvAb-2441_G6_31_Ang2k_LC08	1392	184
TvAb-2441bevacizumab_LC06	2831	503
TvAb-2441_bevacizumab_LC08	2305	972
TvAb-<ANG-2>Mab 536	>20000	
TvAb-<VEGF>Bevacizumab	1584	357
TvAb-3421_bevacizumab_LC06	2660	284
TvAb-4421_bevacizumab_LC06	1980	1319
TvAb-4461_bevacizumab_LC06	1677	394

Example 8 (reference)

HUVEC proliferation

[0203] In order to confirm that the anti-VEGF related activities were retained in the bispecific tetravalent <VEGF-ANG2> antibodies TvAb-2441-bevacizumab-LC06 and TvAb-2441-bevacizumab-LC08 it was shown that TvAb-2441-bevacizumab-LC06 and TvAb-2441-bevacizumab-LC08 interfere with VEGF-induced HUVEC proliferation in a compa-

rable manner as their mother clones LC06 and LC08 in the VEGF-induced HUVEC proliferation assay as described above.

[0204] Figure 18 shows that indeed TvAb-2441-bevacizumab-LC06 and TvAb-2441-bevacizumab-LC08 interfere in a concentration dependent manner with VEGF-induced HUVEC proliferation comparable to the parental antibody bevacizumab.

Example 9 (reference)

ELISA Binding assay to human ANG-1 and to human ANG-2

[0205] The binding of parent <ANG-2> antibodies Ang2i-LC06, Ang2i-LC07 and Ang2k-LC08 to human ANG-1 and human ANG-2 was determined in an ANG-1 or ANG-2 binding ELISA as described above (see Comparative binding to ANG-1 and ANG-2 (ANG-1 and ANG-2 binding ELISA)). Briefly, the ELISA-type assay is based on the immobilization of human wild-type Angiopoieti-1 or -2 in a microtiter plate. Binding of an antibody directed against the immobilized ANG-1 or ANG-2 is measured via an <human Fc> (anti-IgG) antibody with a POD conjugate. A dilution series of the <ANG-2>antibody allows to determine an EC_{50} concentration. As a reference the human anti-ANG-2 antibody <ANG-2> antibody Mab536 (Oliner et al., Cancer Cell. 2004 Nov;6(5):507-16, US 2006/0122370) was used. The determined EC50 concentrations are summarized in the table below.

Antibody	hANG-1 binding EC50	hANG-2 binding EC50
<ANG-2>MAb536	2538 ng/mL	133 ng/mL
<ANG-2>Ang2i-LC06	> 8000 ng/mL	84 ng/mL
<ANG-2>Ang2i-LC07	> 8000 ng/mL	3006 ng/mL
<ANG-2>Ang2i-LC08	4044 ng/mL	105 ng/mL

[0206] All antibodies are specifically binding to ANG-2. MAb536 and Ang2k-LC08 show also specific binding towards ANG-1, whereas Ang2i-LC06 and Ang2i-LC07 are not specifically binding to ANG-1 as they have an EC50-value of above 8000 ng/ml (detection limit).

Example 10 (reference)

Expression & Purification of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecules scFAb-Avastin-LC06-2620, scFab-Avastin-Ang2i-LC06-2640 and scFab-Avastin-Ang2i-LC06-2641

[0207] Analogous to the procedures described in Example 1 and in the materials and methods above, the bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecules scFAb-Avastin- LC06-2620, scFab-Avastin- LC06-2640 and scFab-Avastin- LC06-2641, all three based on <VEGF> bevacizumab and <ANG-2> Ang2i-LC06 were expressed and purified. Binding affinities and other properties were determined as described in the the Examples above. The relevant (eventually modified) light and heavy chains amino acid sequences of these bispecific antibodies are given in SEQ ID NO: 109 -110 (scFAb-Avastin-LC06-2620), in SEQ ID NO: 111 -112 (scFAb-Avastin-LC06-2640) and in SEQ ID NO: 113-114 (scFAb-Avastin-LC06-2641).

Key data	scFAb-Avastin-LC06-2620	scFAb-Avastin-Ang2i-LC06-2640	scFAb-Avastin-LC06-Ang2i-2641
Expression (Yield)	29µg/mL	27 µg/mL	18 µg/mL
Purification (Yield, Prot. A. homog.)	21 mg, 57%	19 mg, 86%	12 mg, 90%
Homogeneity after preparative SEC	98%	98%	99%
Function			
hANG-2 affinity (Biacore)	1.9 E-9 M	1.8 E-9 M	1.9 E-9 M
hVEGF affinity (Biacore)	1 E-10 M	1E-10 M	1E-10 M

Example 11 (reference)

Expression & Purification of bispecific, trivalent single chain Fab <VEGF-ANG-2> antibody molecule Avastin-LC06-KiH-C-scFab

[0208] Analogous to the procedures described in Example 1 and in the materials and methods above, the bispecific, trivalent single chain Fab <VEGF-ANG-2> antibody molecule Avastin-LC06-KiH-C-scFab based on <VEGF> bevacizumab and <ANG-2> Ang2i-LC06 were expressed and purified. Binding affinities and other properties were determined as described in the the Examples above. The relevant (eventually modified) light and heavy chains amino acid sequences of this bispecific antibody are given in SEQ ID NO: 115-117 (Avastin-LC06-KiH-C-scFab).

Key data	Avastin-LC06-KiH-C-scFab
Expression (Yield)	15 µg/mL
Purification (Yield, Prot. A. homog.)	4,8 mg, 91 %
Homogeneity after preparative SEC	97%
Function	
hANG-2 affinity (Biacore)	4.4 E-9 M
hVEGF affinity (Biacore)	1 E-10 M

Example 12 (reference)

Expression & Purification of bispecific, trivalent <VEGF-ANG-2> antibody molecule Avastin-LC06-C-Fab-6CSS

[0209] Analogous to the procedures described in Example 1 and in the materials and methods above (see also , the bispecific, trivalent <VEGF-ANG-2> antibody molecule Avastin-LC06-C-Fab-6CSS based on <VEGF> bevacizumab and <ANG-2> Ang2i-LC06 were expressed and purified. Binding affinities and other properties were determined as described in the the Examples above. Bispecific, trivalent antibody molecules of this format in general are described in EP Appl. No 09005108.7. The relevant (eventually modified) light and heavy chains amino acid sequences of this bispecific <VEGF-ANG-2> antibody are given in SEQ ID NO: 118-120 (Avastin-LC06-C-Fab-6CSS).

Key data	scFAb-Avastin-LC06-2620	scFAb-Avastin-LC06-2640	scFAb-Avastin-LC06-2641
Expression (Yield)	29µg/mL	27 µg/mL	18 µg/mL
Purification (Yield, Prot. A. homog.)	21 mg, 57%	19 mg, 86%	12 mg, 90%
Homogeneity after preparative SEC	98%	98%	99%
Function			
hANG-2 affinity (Biacore)	1.9 E-9 M	1.8 E-9 M	1.9 E-9 M
hVEGF affinity (Biacore)	1 E-10 M	1E-10 M	1E-10 M

Example 13

Expression & Purification of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecules Avastin-LC06-CH1-CL, Avastin-LC06-VH-VL and Avastin-LC06-VH-VL-SS

[0210] Analogous to the procedures described in Example 1 and in the materials and methods above, the bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecules Avastin-LC06-CH1-CL (CH-CL exchange as described in WO 2009/080253), Avastin-LC06-VH-VL (VH-VL exchange as described in WO 2009/080252) and Avastin-LC06-VH-VL-SS (VH-VL exchange as described in WO 2009/080252 and an additional introduced VH44 VL100 disulfide brigde) based on <VEGF> bevacizumab and <ANG-2> Ang2i-LC06 were expressed and purified. Binding affinities and

other properties were determined as described in the the Examples above. The relevant (eventually modified) light and heavy chains amino acid sequences of these bispecific antibodies are given in SEQ ID NO: 121 -124 (Avastin-LC06-CH1-CL), in SEQ ID NO: 125 -128 (Avastin-LC06-VH-VL) and in SEQ ID NO: 129 -132 (Avastin-LC06-VH-VL-SS).

Key data	Avastin-LC06-CM-CHl-CL	Avastin-LC06-CM-VH-VL	Avastin-LC06-VH-VL-SS
Expression (Yield)	87 µg/mL	44 µg/mL	65 µg/mL
Purification (Yield, Prot. A. homog.)	50 mg, 62%	22 mg, 95%	91 mg, 74%
Homogeneity after preparative SEC	84%	> 99 %	95 %
Function			
hANG-2 affinity (Biacore)	1.3 E-9 M	2.1 E-9 M	1.46 E-9 M
hVEGF affinity (Biacore)	1 E-10 M	1 E-10 M	1 E-10 M

Example 14

Expression & Purification of bispecific, bivalent ScFab-Fc fusion <VEGF-ANG-2> antibody molecules Avastin-LC06-N-scFab and Avastin-LC06-N-scFabSS

[0211] Analogous to the procedures described in Example 1 and in the materials and methods above, the bispecific, bivalent ScFab-Fc fusion <VEGF-ANG-2> antibody molecules Avastin-LC06-N-scFab and Avastin-LC06-N-scFabSS based on <VEGF> bevacizumab and <ANG-2> Ang2i-LC06 were expressed and purified.. Binding affinities and other properties were determined as described in the the Examples above. The relevant modified heavy chains amino acid sequences of these bispecific antibodies are given in SEQ ID NO: 133-134 (Avastin-LC06-N-scFab), and in SEQ ID NO: 135 -136 (Avastin-LC06-N-scFabSS).

Key data	Avastin-LC06-N-scFab	Avastin-LC06-N-scFabSS
Expression (Yield)		62 µg/mL
Purification (Yield, Prot. A. homog.)		43 %
Function		
hANG-2 affinity (Biacore)		1 nM
VEGF affinity (Biacore)		1 nM

Example 15

Inhibition of hVEGF binding to hVEGF Receptor (ELISA), Blocking of VEGF-induced tube formation, Inhibition of huANG-2 binding to Tie-2 (ELISA), Tie2 phosphorylation, and HUVEC proliferation of the bispecific, <VEGF-ANG-2> antibody molecules of Examples 10 to 14

[0212] Inhibition of hVEGF binding to hVEGF Receptor (ELISA), blocking of VEGF-induced tube formation, Inhibition of huANG-2 binding to Tie-2 (ELISA), Tie2 phosphorylation, and HUVEC proliferation of the bispecific, <VEGF-ANG-2> antibody molecules of Examples 10 to 14 can be determined analogously to the procedures described in Materials and Methods and Examples 4 to 9 above.

Example 16 (reference)

In vivo efficacy of bispecific antibody <VEGF-ANG-2> antibody in comparison to <ANG-2> ANG2i-LC06, and the combination of <ANG-2> ANG2i-LC06 and Avastin in the refractory Colo205 xenograft model in Scid beige mice (after resistance to bevacizumab (Avastin) treatment)

Cell lines and culture conditions:

[0213] Colo205 human colorectal cancer cells were originally obtained from ATCC and after expansion deposited in the Roche Penzberg internal cell bank. Tumor cell line was routinely cultured in RPMI 1640 medium (PAA, Laboratories, Austria) supplemented with 10% fetal bovine serum (PAA Laboratories, Austria) and 2 mM L-glutamine, at 37°C in a water-saturated atmosphere at 5% CO_2. Passage 2-5 was used for transplantation.

Animals:

[0214] Female SCID beige mice; age 4-5 weeks at arrival (purchased from Charles River Germanyd) were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government. After arrival animals were maintained in the quarantine part of the animal facility for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on regular basis. Diet food (Provimi Kliba 3337) and water (acidified pH 2.5-3) were provided ad libitum. Age of mice at start of the study was about 10 weeks.

Tumor cell injection:

[0215] At the day of injection, tumor cells were harvested (trypsin-EDTA) from culture flasks (Greiner) and transferred into 50 ml culture medium, washed once and resuspended in PBS. After an additional washing step with PBS and filtration (cell strainer; Falcon 0 100µm) the final cell titer was adjusted to 2.5 x 10^7 / ml. Tumor cell suspension was carefully mixed with transfer pipette to avoid cell aggregation. After this, cell suspension was filled into a 1.0 ml tuberculin syringe (Braun Melsungen) using a wide needle (1.10 x 40 mm); for injection needle size was changed (0.45 x 25 mm) and for every injection a new needle was used. Anesthesia was performed using a Stephens inhalation unit for small animals with preincubation chamber (plexiglas), individual mouse nose-mask (silicon) and not flammable or explosive anesthesia compound Isoflurane (cp-pharma) in a closed circulation system. Two days before injection coat of the animals were shaved and for cell injection skin of anaesthetized animals was carefully lifted up with an anatomic forceps and 100 µl cell suspension (= 2.5 x 10^6 cells) was injected subcutaneously in the right flank of the animals.

Treatment of animals

Pretreatment:

[0216] Animal treatment started 14 days after cell transplantation (study Ang2_PZ_Colo205_008) at a mean tumor volume of 100 mm^3 to 150 mm^3, respectively. Mice were treated once weekly with Avastin (10 mg/kg) for a time period of 5 weeks.

Secondary treatment:

[0217] Thereafter mice were randomized for 2nd treatment and divided to four groups with 10 mice in each group. Tumor volume at start of secondary treatment at day 51 was in the range from 336 to 341 mm^3. Mice were treated once weekly i.p. with the different compounds as indicated in following table.

Group	No of animals	Compound	Dose (mg/kg) (nMol/kg)	Route/Mode of administration	No of treatments	Cumulative dose (mg/kg)
11	10	Avastin	10 mg/kg (68 nMol/kg)	i.p. once weekly	11	110
12	10	ANG2i-LC06	10 mg/kg (68 nMol/kg)	i.p. once weekly	6	60

(continued)

Group	No of animals	Compound	Dose (mg/kg) (nMol/kg)	Route/Mode of administration	No of treatments	Cumulative dose (mg/kg)
13	10	ANG2i-LC06 + Avastin	10 mg/kg (68 nMol/kg) + 10 mg/kg (68 nMol/kg)	i.p. once weekly i.p. once weekly	6 11	60 110
14	10	TvAb-2441-bevacizumab-LC06	13 mg/kg (64 nMol/kg)	i.p. once weekly	6	78

Monitoring:

[0218] Animals were controlled 2x per week for their health status. Body weights were documented 2x per week after cell injection. The tumor dimensions were measured by caliper beginning on the staging day and subsequently 2 times per week during the whole treatment period. Tumor volume was calculated according to NCI protocol (Tumor weight = $1/2ab^2$, where "a" and "b" are the long and the short diameters of the tumor, respectively). Termination criteria were the critical tumor mass (up to 1.7 g or Ø > 1.5 cm), body weight loss more than 20% from baseline, tumor ulceration or poor general condition of the animals.

Results: Tumor growth inhibition based on medians (in percent) at day 91

[0219]

	TGI
ANG2i-LC06 10 mg/kg (68 nMol/kg) i.p.; Avastin 10 mg/kg (68 nMol/kg) i.p.	45.3
ANG2i-LC06 10 mg/kg i.p. (68 nMol/kg)	44.4
TvAb-2441-bevacizumab-LC06_13mg/kg i.p. (64 nMol/kg)	60.4

[0220] The results show that the bispecific <VEGF-ANG-2> antibody TvAb-2441-bevacizumab-LC06 showed a higher tumor growth inhibition (at lower doses) in the bevacizumab(Avastin)- resistant xenograft tumor model Colo205 in Scid beige mice compared to the treatment with monospecific antibody ANG2i-LC06 alone or the combination of ANG2i-LC06 and Avastin.

Examples 17 (reference)

In vivo inhibition of tumor angiogenesis in s.c. Calu-3 NSCLC xenograft

- Detection via non-invasive in vivo imaging of angiogenesis using anti-CD31 labeled with

Cell lines and culture conditions:

[0221] This human lung adenocarcinoma cancer cell line has been established from a human caucasian male with lung cancer. Cells were obtained from Roche, Kamakura and passaged in house for working cell bank. Tumor cells are routinely cultured in RPMI1640 medium (PAN Biotech, Germany) supplemented with 10% fetal bovine serum (PAN Biotech, Germany) and 2 mM L-glutamine (PAN Biotech, Germany) at 37°C in a water-saturated atmosphere at 5% CO_2. Culture passage is performed with trypsin / EDTA 1x (PAN) splitting one time / week.

Animals:

[0222] Female BALB/c nude mice; age 4-5 weeks at arrival (purchased from Charles River Germany) were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government. After

arrival animals were maintained in the quarantine part of the animal facility for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on regular basis. Diet food (Provimi Kliba 3337) and water (acidified pH 2.5-3) were provided ad libitum. Age of mice at start of the study was about 10 weeks.

Tumor cell injection:

[0223] At the day of injection, tumor cells were harvested (trypsin-EDTA) from culture flasks (Greiner) and transferred into 50 ml culture medium, washed once and resuspended in PBS. After an additional washing step with PBS and filtration (cell strainer; Falcon 0 100µm) the final cell titer was adjusted to 5.0 x 10^7/ml. Tumor cell suspension was carefully mixed with transfer pipette to avoid cell aggregation. After this, cell suspension was filled into a 1.0 ml tuberculin syringe (Braun Melsungen) using a wide needle (1.10 x 40 mm); for injection needle size was changed (0.45 x 25 mm) and for every injection a new needle was used. Anesthesia was performed using a Stephens inhalation unit for small animals with preincubation chamber (plexiglas), individual mouse nose-mask (silicon) and not flammable or explosive anesthesia compound Isoflurane (cp-pharma) in a closed circulation system. Two days before injection coat of the animals were shaved and for cell injection skin of anaesthetized animals was carefully lifted up with an anatomic forceps and 100 µl cell suspension (= 5.0 x 10^6 cells) was injected subcutaneously in the right flank of the animals.

Treatment of animals

[0224] At study day 35, mice were randomized to statistically well distributed groups, depending on their body weight and tumor size. For the treatment with therapeutic antibodies, each group consisted of 10 mice and treatment with therapeutic antibodies was applied once weekly i.p. for a 6 week time period. (see Fig 19)

Group 1:	vehicle (Xolair) 10 mg/kg
Group 2:	Avastin 10 mg/kg
Group 3:	Combination of monospecific <VEGF> Avastin 10 mg/kg plus monospecific <ANG-2> Ang2i-LC06 10 mg/kg (= Avastin / Ang2i-LC06)
Group 4:	Bispecific <VEGF-ANG-2> antibody 2441-Avastin-scFv-LC06 13.3 mg/kg

Monitoring:

[0225] Animals were controlled 2x per week for their health status. Body weights were documented 2x per week after cell injection. The tumor dimensions were measured by caliper beginning on the staging day and subsequently 2 times per week during the whole treatment period. Tumor volume was calculated according to NCI protocol (Tumor weight = $1/2ab^2$, where "a" and "b" are the long and the short diameters of the tumor, respectively). Termination criteria were the critical tumor mass (up to 1.7 g or Ø > 1.5 cm), body weight loss more than 20% from baseline, tumor ulceration or poor general condition of the animals.

[0226] Blood vessel and angiogenesis monitoring with labeled anti-CD 31 antibody

[0227] Preliminary studies revealed that anti-CD31 antibody as best agent for imaging tumor vasculature. This agent targets mouse endothelial CD31 receptors and visualizes single blood vessels with a low signal-to-background ratio. Therefore, imaging for anti-CD31 antibody represents a feasible way to image tumor vasculature. Three mice of each therapy group have been chosen and injected i.v. with 50 µg/mouse anti-CD3 antibody labeled covalently with the organic fluorophore Alexa610 at day 35, 49 and 79. Near-infrared imaging was carried out 24 hrs after each application of the labeled antibody under inhalation anesthesia. An increase or decrease of tumor vasculature was visualized by using the compare image tool of the MAESTRO system. Under treatment with the control mab Xolair and the therapeutic antibody Avastin an increase of tumor blood vessels from day 35 to day 79 was observed. In contrast, the combined treatment with Avastin plus Ang2i-LC06 and 2441-Avastin-scFv-LC06 exhibited a decrease of tumor vasculature (Fig 19).

[0228] Tumor regions were quantified by manually drawing measurement areas and signal intensities were evaluated in intensity values (total signal / exposure time). The average changes of CD31 signals from day 35 to 49 and from day 49 to 79 were plotted in Fig 19. All treatment groups revealed an increase in tumor vasculature from day 35 to 49. While CD31 tumor signals steadily accelerated in group 1 (Xolair) and group 2 (Avastin), tumor vasculature significantly decreased in group 3 (Combination of Avastin plus <ANG-2> Ang2i-LC06) and group 4 (Bispecific <VEGF-ANG-2> antibody 2441-Avastin-scFv-LC06), with group 4 showing clearly the most pronounced antiangiogenic effect (Fig 19).

[0229] Immediately after the last *in vivo* imaging studies, tumors were explantated (day 79), fixed in formalin and embedded in paraffin for ex *vivo* studies. Fluorescence microscopy showed numerous well defined capillaries in tumors treated with control mab Xolair. Several tumor blood vessels were observed in mice treated with Avastin. In contrast, treatment groups 3 and 4 had significantly fewer and less defined blood vessels in the tumors compared to treatment

groups 1 and 2 whereas group 4 showed the most pronounced effect. Group 4 revealed lower microvessel density, capillaries were generally smaller and unstructured and they exhibited weaker anti-CD31 fluorescence signals as Group 1, 2 and 3. Histochemical HE-staining showed intratumoral necrotic regions for up to 90% of all regions in the treatment group with the bispecific antibody of group 4 which is clearly higher than for the other treatment groups (data not shown).

Example 18

In vivo efficacy of bispecific antibodies <VEGF-ANG-2> and compared to the parent monospecific antibodies (alone or in combination) in the staged subcutaneous Colo205 xenograft model in Scid beige mice

Cell lines and culture conditions:

[0230] Colo205 human colorectal cancer cells were originally obtained from ATCC and after expansion deposited in the Roche Penzberg internal cell bank. Tumor cell line was routinely cultured in RPMI 1640 medium (PAA, Laboratories, Austria) supplemented with 10% fetal bovine serum (PAA Laboratories, Austria) and 2 mM L-glutamine, at 37°C in a water-saturated atmosphere at 5% CO_2. Passage 2-5 was used for transplantation.

Animals:

[0231] Female SCID beige mice; age 4-5 weeks at arrival (purchased from Charles River Germany) were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government. After arrival animals were maintained in the quarantine part of the animal facility for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on regular basis. Diet food (Provimi Kliba 3337) and water (acidified pH 2.5-3) were provided ad libitum. Age of mice at start of the study was about 10 weeks.

Tumor cell injection:

[0232] At day of injection Colo205 cells were centrifuged, washed once and resuspended in PBS. After an additional washing with PBS cell concentration and cell size were determined using a cell counter and analyzer system (Vi-CELL, Beckman Coulter). For injection of Colo205 cells, the final titer was adjusted to 5.0 x 10E7 cells/ml, viability ca. 90%. Subsequently 100 µl of this suspension corresponding to 2.5*106 cells per animal was injected s.c. into the right flank of the mice.

[0233] Treatment of animals started at day of randomisation, 16 days after cell transplantation (study Ang2_PZ_Colo205_009)) at a mean tumor volume of 100 mm3, respectively.

[0234] Dose schedule of Study Ang2_PZ_Colo205_009:

No of animals	Compound	Dose (mg/kg)	Route/Mode of administration
10	Xolair	10	i.p. once weekly
10	<VEGF > Avastin	10	i.p. once weekly
10	< ANG-2> Ang2i-LC06	10	i.p. once weekly
10	Ang2i-LC06 + Avastin	10 10	i.p. once weekly i.p.once weekly
10	<VEGF-ANG-2> TvAb-2441-bevacizumab-LC06	13.3	i.p. once weekly
10	<VEGF-ANG-2> Avastin-LC06-CH1-CL	20	i.p. once weekly
10	<VEGF-ANG-2> scFAb-Avastin-LC06-2620	16.6	i.p. once weekly

Monitoring:

[0235] Animals were controlled 2x per week for their health status. Body weights were documented 2x per week after cell injection. The tumor dimensions were measured by caliper beginning on the staging day and subsequently 2 times per week during the whole treatment period. Tumor volume was calculated according to NCI protocol (Tumor weight = $1/2ab^2$, where "a" and "b" are the long and the short diameters of the tumor, respectively).Termination criteria were the

critical tumor mass (up to 1.7 g or Ø > 1.5 cm), body weight loss more than 20% from baseline, tumor ulceration or poor general condition of the animals.

Results:

Tumor growth inhibition (TGI) based on medians (in percent) at day 61

[0236]

	TGI
<VEGF > Avastin	66
< ANG-2> Ang2i-LC06	47
Ang2i-LC06 + Avastin	78
<VEGF-ANG-2> TvAb-2441-bevacizumab-LC06	87
<VEGF-ANG-2> Avastin-LC06- CHI-CL	92
<VEGF-ANG-2> scFAb-Avastin-LC06-2620	86

[0237] The results show that all three bispecific <VEGF-ANG-2> Avastin (bevacizumab)-ANG2i-LC06 antibodies (all based on the bevacizumab sequences SEQ ID No: 7 and 8 and on ANG2i-LC06 sequences SEQ ID No: 52 and 53) showed a higher tumor growth inhibition in xenograft tumor model Colo205 in Scid beige mice compared to the treatment with monospecific antibodies ANG2i-LC06 and Avastin alone or the combination of ANG2i-LC06 and Avastin.

Example 19

Expression & Purification and properties of bispecific <VEGF-ANG-2> antibody molecules scFAb-Avastin-LC10-2620, scFab-Avastin-LC10-2640 and scFab-Avastin-LC10-2641, Avastin-LC10-KiH-C-scFab, Avastin-LC10-C-Fab-6CSS, Avastin-LC10-CHI-CL, Avastin-LC10-VH-VL and Avastin-LC10-VH-VL-SS,Avastin-LC10-N-scFab and Avastin-LC10-N-scFabSS

[0238] By replacing the VH and VL domains of Ang2i-LC06 (SEQ ID No: 52 and 53) with the corresponding VH and VL domains of Ang2i-LC10 (SEQ ID No: 84 and 85) and using the (apart from such replacement) analogous procedures and sequences described in Example 10 to 14, the bispecific, <VEGF-ANG-2> antibody molecules scFAb-Avastin-LC10-2620, scFab-Avastin-LC10-2640 and scFab-Avastin-LC10-2641, Avastin-LC10-KiH-C-scFab, Avastin-LC10-C-Fab-6CSS, Avastin-LC 10-CHI-CL, Avastin-LC 10-VH-VL and Avastin-LC 10-VH-VL-SS,Avastin-LC10-N-scFab and Avastin-LC10-N-scFabSS, all based on <VEGF> bevacizumab and <ANG-2> Ang2i-LC10 are expressed and purified.

[0239] Binding affinities and other in vitro properties are determined as described in the the Examples above.

Example 20

In vivo efficacy of bispecific antibody <VEGF-ANG-2> molecules scFAb-Avastin-LC10-2620, scFab-Avastin-LC10-2640 and scFab-Avastin-LC10-2641, Avastin-LC10-KiH-C-scFab, Avastin-LC10-C-Fab-6CSS, Avastin-LC10-CHI-CL, Avastin-LC10-VH-VL and Avastin-LC10-VH-VL-SS,Avastin-LC10-N-scFab and Avastin-LC10-N-scFabSS.

[0240] In vivo efficacy of bispecific antibody <VEGF-ANG-2> molecules scFAb-Avastin-LC10-2620, scFab-Avastin-LC10-2640 and scFab-Avastin-LC10-2641, Avastin-LC 10-KiH-C-scFab, Avastin-LC10-C-Fab-6CSS, Avastin-LC 10-CHI -CL, Avastin-LC10-VH-VL and Avastin-LC10-VH-VL-SS,Avastin-LC10-N-scFab and Avastin-LC10-N-scFabSS is determined analogously to the corresponding Examples above.

SEQUENCE LISTING

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<150> EP 08017607.6 <151> 2008-10-08

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65                  70                  75                  80

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                85                  90                  95

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Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

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<400> 25

Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Tyr Gly Tyr
1 5 10

<210> 26
<211> 17
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR2, <ANG-2> Mab 536

<400> 26

Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 27
<211> 5
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR1, <ANG-2> Mab 536

<400> 27

Ser Tyr Gly Met His
1 5

<210> 28
<211> 9
<212> PRT
<213> Artificial

<220>
<223> light chain CDR3, <ANG-2> Mab 536

<400> 28

Met Gln Gly Thr His Trp Pro Pro Thr
1 5

<210> 29
<211> 7
<212> PRT
<213> Artificial

<220>
<223> light chain CDR2, <ANG-2> Mab 536

<400> 29

Leu Gly Ser Asn Arg Ala Ser
1 5

<210> 30
<211> 16
<212> PRT
<213> Artificial

<220>
<223> light chain CDR1, <ANG-2> Mab 536

<400> 30

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp
1 5 10 15

<210> 31
<211> 122
<212> PRT
<213> Artificial

<220>
<223> heavy chain variable domain, <ANG-2> Mab 536

<400> 31

Glu Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Tyr Gly Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 32
<211> 112
<212> PRT
<213> Artificial

<220>
<223> light chain variable domain, <ANG-2> Mab 536

<400> 32

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
85 90 95

Thr His Trp Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 33
<211> 20
<212> PRT
<213> Artificial

<220>
<223> (G4S)4 linker

<400> 33

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser
20

<210> 34
<211> 104
<212> PRT
<213> Homo sapiens

<400> 34

Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
1 5 10 15

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
20 25 30

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
35 40 45

Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
50 55 60

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
65 70 75 80

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
85 90 95

Thr Val Ala Pro Thr Glu Cys Ser
100

<210> 35
<211> 330

<212> PRT
<213> Homo sapiens

<400> 35

```
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                   10                  15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
```

180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225 230 235 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 36
<211> 327
<212> PRT
<213> Homo sapiens

<400> 36

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

```
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro
            100                 105                 110

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
        115                 120                 125

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
    130                 135                 140

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145                 150                 155                 160

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
                165                 170                 175

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
            180                 185                 190

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
        195                 200                 205

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
    210                 215                 220

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225                 230                 235                 240

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
                245                 250                 255

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
            260                 265                 270
```

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
275 280 285

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
290 295 300

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305 310 315 320

Leu Ser Leu Ser Leu Gly Lys
325

<210> 37
<211> 107
<212> PRT
<213> Homo sapiens

<400> 37

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1 5 10 15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65 70 75 80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 38
<211> 7
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR3, <ANG-2> Ang2s_R3_LC03

<400> 38

Asp Leu Gly Tyr Asp Tyr Val
1 5

<210> 39
<211> 19
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR2, <ANG-2> Ang2s_R3_LC03

<400> 39

Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala Pro
1 5 10 15

Val Lys Gly

<210> 40
<211> 5
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR1, <ANG-2> Ang2s_R3_LC03

<400> 40

Asn Ala Trp Met Ser
1 5

<210> 41
<211> 10
<212> PRT
<213> Artificial

<220>
<223> light chain CDR3, <ANG-2> Ang2s_R3_LC03

<400> 41

Gln Gln Tyr Asp Asn Leu Pro Met Tyr Thr
1 5 10

<210> 42
<211> 7
<212> PRT
<213> Artificial

<220>
<223> light chain CDR2, <ANG-2> Ang2s_R3_LC03

<400> 42

His Ala Ser Asn Leu Glu Thr
1 5

<210> 43
<211> 11
<212> PRT
<213> Artificial

<220>
<223> light chain CDR1, <ANG-2> Ang2s_R3_LC03

<400> 43

```
Gln Ala Ser Gln Asp Ile Ser Asn Arg Leu Asn
1               5                   10
```

<210> 44
<211> 118
<212> PRT
<213> Artificial

<220>
<223> heavy chain variable domain, <ANG-2> Ang2s_R3_LC03

<400> 44

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
    50                  55                  60

Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
                85                  90                  95

Tyr Cys Thr Thr Asp Leu Gly Tyr Asp Tyr Val Trp Gly Gln Gly Thr
            100                 105                 110

Leu Val Thr Val Ser Ser
        115
```

<210> 45
<211> 110
<212> PRT
<213> Artificial

<220>

<223> light chain variable domain, <ANG-2> Ang2s_R3_LC03

<400> 45

```
Asp Ile Gln Val Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Arg
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr His Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Asn Leu Pro Met
                85                  90                  95

Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
            100                 105                 110
```

<210> 46
<211> 20
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR3, <ANG-2>Ang2i_LC06

<400> 46

```
Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr Pro Gly
1               5                  10                  15

Ala Phe Asp Ile
            20
```

<210> 47
<211> 17
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR2, <ANG-2>Ang2i_LC06

<400> 47

```
Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe Gln
1               5                  10                  15

Gly
```

<210> 48
<211> 5
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR1, <ANG-2>Ang2i_LC06

<400> 48

```
Gly Tyr Tyr Met His
1               5
```

<210> 49
<211> 11
<212> PRT
<213> Artificial

<220>
<223> light chain CDR3, <ANG-2>Ang2i_LC06

<400> 49

```
Gln Val Trp Asp Ser Ser Ser Asp His Tyr Val
1               5                  10
```

<210> 50
<211> 7
<212> PRT
<213> Artificial

<220>
<223> light chain CDR2, <ANG-2>Ang2i_LC06

<400> 50

```
Asp Asp Ser Asp Arg Pro Ser
1               5
```

<210> 51
<211> 11
<212> PRT
<213> Artificial

<220>
<223> light chain CDR1, <ANG-2>Ang2i_LC06

<400> 51

```
Gly Gly Asn Asn Ile Gly Ser Lys Ser Val His
1               5                  10
```

<210> 52
<211> 129
<212> PRT
<213> Artificial

<220>
<223> heavy chain variable domain, <ANG-2>Ang2i_LC06

<400> 52

```
Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys

                85                  90                  95

Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110

Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125

Ser
```

<210> 53
<211> 110
<212> PRT
<213> Artificial

<220>
<223> light chain variable domain, <ANG-2>Ang2i_LC06

<400> 53

```
Gln Pro Gly Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1               5                   10                  15

Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45

Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80

Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95

Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Gln
            100                 105                 110
```

<210> 54
<211> 20
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR3, <ANG-2>Ang2i_LC07

<400> 54

```
Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr Pro Gly
1               5                   10                  15

Ala Phe Asp Ile
            20
```

<210> 55
<211> 17
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR2, <ANG-2>Ang2i_LC07

<400> 55

```
Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe Gln
1               5                   10                  15

Gly
```

<210> 56
<211> 5
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR1, <ANG-2>Ang2i_LC07

<400> 56

Gly Tyr Tyr Met His
1 5

<210> 57
<211> 11
<212> PRT
<213> Artificial

<220>
<223> light chain CDR3, <ANG-2>Ang2i_LC07

<400> 57

Gln Val Trp Asp Ser Asp Ser Asp Gln Gly Val
1 5 10

<210> 58
<211> 7
<212> PRT
<213> Artificial

<220>
<223> light chain CDR2, <ANG-2>Ang2i_LC07

<400> 58

Asp Asp Ser Glu Arg Pro Ser
1 5

<210> 59
<211> 11
<212> PRT
<213> Artificial

<220>
<223> light chain CDR1, <ANG-2>Ang2i_LC07

<400> 59

Gly Gly Asn Phe Ile Gly Gly Lys Ser Val His
1 5 10

<210> 60
<211> 129
<212> PRT

<213> Artificial

<220>
<223> heavy chain variable domain, <ANG-2>Ang2i_LC07

<400> 60

```
Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110

Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125

Ser
```

<210> 61
<211> 110
<212> PRT
<213> Artificial

<220>
<223> light chain variable domain, <ANG-2>Ang2i_LC07

<400> 61

```
Gln Pro Val Leu Thr Gln Ser Pro Ser Val Ser Val Ala Pro Gly Glu
1               5                  10                  15

Thr Ala Arg Val Ala Cys Gly Gly Asn Phe Ile Gly Gly Lys Ser Val
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35                  40                  45

Asp Asp Ser Glu Arg Pro Ser Gly Ile Pro Glu Arg Ile Ser Gly Ser
    50                  55                  60

Asn Ser Gly Asn Thr Ala Thr Leu Ile Ile Thr Arg Ala Glu Ala Gly
65                  70                  75                  80

Asp Glu Ala Asp Tyr His Cys Gln Val Trp Asp Ser Asp Ser Asp Gln
                85                  90                  95

Gly Val Phe Gly Thr Gly Thr Lys Leu Thr Val Leu Gly Gln
            100                 105                 110
```

<210> 62
<211> 15
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR3, <ANG-2>Ang2k_LC08

<400> 62

```
Pro Thr Leu Asp Ile Tyr Met Gly Tyr Tyr Tyr Gly Met Asp Val
1               5                  10                  15
```

<210> 63
<211> 17
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR2, <ANG-2>Ang2k_LC08

<400> 63

```
Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1               5                  10                  15

Gly
```

<210> 64
<211> 5
<212> PRT

<213> Artificial

<220>
<223> heavy chain CDR1, <ANG-2>Ang2k_LC08

<400> 64

```
Ser Tyr Gly Met His
1               5
```

<210> 65
<211> 11
<212> PRT
<213> Artificial

<220>
<223> light chain CDR3, <ANG-2>Ang2k-LCO8

<400> 65

```
Ala Ala Trp Asp Asp Ser Leu Asn Gly Pro Val
1               5                   10
```

<210> 66
<211> 7
<212> PRT
<213> Artificial

<220>
<223> light chain CDR2, <ANG-2>Ang2k_LC08

<400> 66

```
Asn Asn Asp Gln Arg Pro Ser
1               5
```

<210> 67
<211> 13
<212> PRT
<213> Artificial

<220>
<223> light chain CDR1, <ANG-2>Ang2k_LC08

<400> 67

```
Ser Gly Phe Ala Ser Asn Ile Gly Ser Asn Ser Val Asn
1               5                   10
```

<210> 68
<211> 124
<212> PRT
<213> Artificial

<220>
<223> heavy chain variable domain, <ANG-2> Ang2k_LC08

<400> 68

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Pro Thr Leu Asp Ile Tyr Met Gly Tyr Tyr Tyr Gly Met Asp
            100                 105                 110

Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120
```

<210> 69
<211> 112
<212> PRT
<213> Artificial

<220>
<223> light chain variable domain, <ANG-2> Ang2k_LC08

<400> 69

```
Gln Pro Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Ala Pro Gly Gln
1               5                  10                  15

Arg Val Thr Ile Ser Cys Ser Gly Phe Ala Ser Asn Ile Gly Ser Asn
            20                  25                  30

Ser Val Asn Trp Tyr Gln Gln Val Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45

Ile Tyr Asn Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Arg Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65                  70                  75                  80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85                  90                  95

Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
            100                 105                 110
```

<210> 70
<211> 7
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR3, <ANG-2> Ang2s_LC09

<400> 70

```
Asp Leu Gly Tyr Asp Tyr Val
1               5
```

<210> 71
<211> 19
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR2, <ANG-2> Ang2s_LC09

<400> 71

```
Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala Pro
1               5                  10                  15

Val Lys Gly
```

<210> 72
<211> 5
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR1, <ANG-2> Ang2s_LC09

<400> 72

Asn Ala Trp Met Ser
1 5

<210> 73
<211> 7
<212> PRT
<213> Artificial

<220>
<223> light chain CDR3, <ANG-2> Ang2s_LC09

<400> 73

Met Gln Ala Leu Gln Ile Pro
1 5

<210> 74
<211> 7
<212> PRT
<213> Artificial

<220>
<223> light chain CDR2, <ANG-2> Ang2s_LC09

<400> 74

Leu Gly Ser Asn Arg Ala Ser
1 5

<210> 75
<211> 16
<212> PRT
<213> Artificial

<220>
<223> light chain CDR1, <ANG-2> Ang2s_LC09

<400> 75

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp
1 5 10 15

<210> 76
<211> 119
<212> PRT
<213> Artificial

<220>
<223> heavy chain variable domain, <ANG-2> Ang2s_LC09

<400> 76

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
    50                  55                  60

Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                  70                  75                  80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
                85                  90                  95

Tyr Cys Thr Thr Asp Leu Gly Tyr Asp Tyr Val Trp Gly Ser Pro Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser
        115
```

<210> 77
<211> 109
<212> PRT
<213> Artificial

<220>
<223> light chain variable domain, <ANG-2> Ang2s_LC09

<400> 77

Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile
1 5 10 15

Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr
20 25 30

Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile
35 40 45

Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala
65 70 75 80

Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Ile Pro Phe
85 90 95

Thr Phe Gly Pro Gly Thr Lys Val Thr Val Leu Arg Thr
100 105

<210> 78
<211> 20
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR3, <ANG-2> Ang2i_LC10

<400> 78

Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr Pro Gly
1 5 10 15

Ala Phe Asp Ile
20

<210> 79
<211> 17
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR2, <ANG-2> Ang2i_LC10

<400> 79

Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 80
<211> 5
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR1, <ANG-2> Ang2i_LC10

<400> 80

```
Gly Tyr Tyr Met His
1               5
```

<210> 81
<211> 11
<212> PRT
<213> Artificial

<220>
<223> light chain CDR3, <ANG-2> Ang2i_LC10

<400> 81

```
Gln Val Trp Asp Ser Ser Ser Asp His Trp Val
1               5                   10
```

<210> 82
<211> 7
<212> PRT
<213> Artificial

<220>
<223> light chain CDR2, <ANG-2> Ang2i_LC10

<400> 82

```
Asp Asp Ser Asp Arg Pro Ser
1               5
```

<210> 83
<211> 11
<212> PRT
<213> Artificial

<220>
<223> light chain CDR1, <ANG-2> Ang2i_LC10

<400> 83

```
Gly Gly Asn Asn Ile Gly Ser Lys Ser Val His
1               5                   10
```

<210> 84
<211> 129
<212> PRT
<213> Artificial

<220>

<223> heavy chain variable domain, <ANG-2> Ang2i_LC10

<400> 84

```
Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr

65                  70                  75                  80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110

Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125

Ser
```

<210> 85
<211> 105
<212> PRT
<213> Artificial

<220>
<223> light chain variable domain, <ANG-2> Ang2i_LC10

<400> 85

```
Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln Thr Ala Arg Ile Thr
1               5                   10                  15

Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val His Trp Tyr Gln Gln
            20                  25                  30

Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr Asp Asp Ser Asp Arg
        35                  40                  45

Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser Asn Ser Gly Asn Thr
    50                  55                  60

Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly Asp Glu Ala Asp Tyr
65                  70                  75                  80

Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His Trp Val Phe Gly Gly
                85                  90                  95

Gly Thr Lys Leu Thr Val Leu Gly Gln
            100                 105
```

<210> 86
<211> 15
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR3, <ANG-2> Ang2k_LC11

<400> 86

```
Pro Thr Leu Asp Ile Tyr Met Gly Tyr Tyr Tyr Gly Met Asp Val
1               5                   10                  15
```

<210> 87
<211> 17
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR2, <ANG-2> Ang2k_LC11

<400> 87

```
Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1               5                   10                  15

Gly
```

<210> 88
<211> 5
<212> PRT

<213> Artificial

<220>
<223> heavy chain CDR1, <ANG-2> Ang2k_LC11

<400> 88

Ser Tyr Gly Met His
1 5

<210> 89
<211> 12
<212> PRT
<213> Artificial

<220>
<223> light chain CDR3, <ANG-2> Ang2k_LC11

<400> 89

Gln Val Trp Asp Ser Ser Ser Asp His Pro Gly Val
1 5 10

<210> 90
<211> 7
<212> PRT
<213> Artificial

<220>
<223> light chain CDR2, <ANG-2> Ang2k_LC11

<400> 90

Asp Asp Ser Asp Arg Pro Ser
1 5

<210> 91
<211> 11
<212> PRT
<213> Artificial

<220>
<223> light chain CDR1, <ANG-2> Ang2k_LC11

<400> 91

Gly Gly Asn Asn Ile Gly Ser Lys Ser Val His
1 5 10

<210> 92
<211> 124
<212> PRT
<213> Artificial

<220>

<223> heavy chain variable domain, <ANG-2> Ang2k_LC11

<400> 92

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Pro Thr Leu Asp Ile Tyr Met Gly Tyr Tyr Tyr Gly Met Asp
            100                 105                 110

Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120
```

<210> 93
<211> 106
<212> PRT
<213> Artificial

<220>
<223> light chain variable domain, <ANG-2> Ang2k_LC11

<220>
<221> misc feature
<222> (98)..(98)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (102)..(102)
<223> Xaa can be any naturally occurring amino acid

<400> 93

```
Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln Thr Ala Arg Ile Thr
1               5                   10                  15

Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val His Trp Tyr Gln Gln
            20                  25                  30

Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr Asp Asp Ser Asp Arg
        35                  40                  45

Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser Asn Ser Gly Asn Thr
    50                  55                  60

Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly Asp Glu Ala Asp Tyr
65                  70                  75                  80

Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His Pro Gly Val Phe Gly
                85                  90                  95

Gly Xaa Thr Lys Leu Xaa Val Leu Gly Gln
            100                 105
```

<210> 94
<211> 12
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR3, <VEGF>B20-4.1

<400> 94

```
Trp Gly His Ser Thr Ser Pro Trp Ala Met Asp Tyr
1               5                   10
```

<210> 95
<211> 17
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR2, <VEGF>B20-4.1

<400> 95

```
Ala Ile Trp Pro Phe Gly Gly Tyr Thr His Tyr Ala Asp Ser Val Lys
1               5                   10                  15

Gly
```

<210> 96
<211> 8
<212> PRT
<213> Artificial

<220>
<223> heavy chain CDR1, <VEGF>B20-4.1

<400> 96

Phe Ser Ile Asn Gly Ser Trp Ile
1 5

<210> 97
<211> 9
<212> PRT
<213> Artificial

<220>
<223> light chain CDR3, <VEGF>B20-4.1

<400> 97

Gln Gln Ser Asn Thr Ser Pro Leu Thr
1 5

<210> 98
<211> 8
<212> PRT
<213> Artificial

<220>
<223> light chain CDR2, <VEGF>B20-4.1

<400> 98

Tyr Ala Ala Ser Asn Leu Ala Ser
1 5

<210> 99
<211> 11
<212> PRT
<213> Artificial

<220>
<223> light chain CDR1, <VEGF>B20-4.1

<400> 99

Arg Ala Ser Gln Val Ile Arg Arg Ser Leu Ala
1 5 10

<210> 100
<211> 117
<212> PRT
<213> Artificial

<220>
<223> heavy chain variable domain, <VEGF>B20-4.1

<400> 100

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Ile Asn Gly Ser
            20                  25                  30

Trp Ile Phe Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Ala Ile Trp Pro Phe Gly Gly Tyr Thr His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Trp Gly His Ser Thr Ser Pro Trp Ala Met Asp Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val
        115
```

<210> 101
<211> 108
<212> PRT
<213> Artificial

<220>
<223> light chain variable domain, <VEGF>B20-4.1

<400> 101

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Val Ile Arg Arg Ser
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ala Ala Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Thr Ser Pro Leu
                85                  90                  95

        Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
            100                 105
```

<210> 102
<211> 730
<212> PRT
<213> Artificial

<220>
<223> bevacizumab heavy chain Ang2i_LC06 scFv fusion peptide of <VEGF-ANG-2> TvAb-2441-bevacizumab-LC06

<400> 102

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
```

```
                    165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400
```

```
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    450                 455                 460

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Glu Ser
465                 470                 475                 480

Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys
                485                 490                 495

Ala Ser Gly Tyr Thr Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln
            500                 505                 510

Ala Pro Gly Gln Cys Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser
        515                 520                 525

Gly Gly Thr Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr
    530                 535                 540

Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg
545                 550                 555                 560

Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro Asn Pro Tyr
                565                 570                 575

Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr Pro Gly Ala Phe Asp Ile Trp
            580                 585                 590

Gly Gln Gly Thr Met Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
        595                 600                 605

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Pro
    610                 615                 620
```

```
Gly Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln Thr Ala
625                 630                 635                 640

Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val His Trp
                645                 650                 655

Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr Asp Asp
            660                 665                 670

Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser Asn Ser
        675                 680                 685

Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly Asp Glu
    690                 695                 700

Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His Tyr Val
705                 710                 715                 720

Phe Gly Cys Gly Thr Lys Val Thr Val Leu
                725                 730
```

<210> 103
<211> 727
<212> PRT
<213> Artificial

<220>
<223> bevacizumab heavy chain Ang2i_LC08 scFv fusion peptide of <VEGF-ANG-2> TvAb-2441-bevacizumab-LC08

<400> 103

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
```

65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
115 120 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
130 135 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145 150 155 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
165 170 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
180 185 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
195 200 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
210 215 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225 230 235 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
290 295 300

```
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    450                 455                 460

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Glu Ser
465                 470                 475                 480

Gly Gly Gly Val Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala
                485                 490                 495

Ala Ser Gly Phe Thr Phe Ser Ser Tyr Gly Met His Trp Val Arg Gln
            500                 505                 510

Ala Pro Gly Lys Cys Leu Glu Trp Val Ala Val Ile Ser Tyr Asp Gly
        515                 520                 525
```

```
Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
    530                 535                 540

Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg
545                 550                 555                 560

Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Pro Thr Leu Asp Ile
                565                 570                 575

Tyr Met Gly Tyr Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr
            580                 585                 590

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
        595                 600                 605

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Pro Val Leu Thr Gln Pro
    610                 615                 620

Pro Ser Ala Ser Gly Ala Pro Gly Gln Arg Val Thr Ile Ser Cys Ser
625                 630                 635                 640

Gly Phe Ala Ser Asn Ile Gly Ser Asn Ser Val Asn Trp Tyr Gln Gln
                645                 650                 655

Val Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Asn Asn Asp Gln Arg
            660                 665                 670

Pro Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Arg Ser Gly Thr Ser
        675                 680                 685

Ala Ser Leu Ala Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala Asp Tyr
    690                 695                 700

Tyr Cys Ala Ala Trp Asp Asp Ser Leu Asn Gly Pro Val Phe Gly Cys
705                 710                 715                 720

Gly Thr Lys Leu Thr Val Leu
                725
```

<210> 104
<211> 214
<212> PRT
<213> Artificial

<220>
<223> light chain of bevacizumab

<400> 104

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

                    Phe Asn Arg Gly Glu Cys
                210
```

<210> 105
<211> 191
<212> PRT
<213> Homo sapiens

<400> 105

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1               5                  10                  15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
            20                  25                  30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
        35                  40                  45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
    50                  55                  60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65                  70                  75                  80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
                85                  90                  95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
            100                 105                 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
        115                 120                 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
    130                 135                 140

Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145                 150                 155                 160

Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
                165                 170                 175

Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg

            180                 185                 190
```

<210> 106
<211> 504
<212> PRT
<213> Artificial

<220>
<223> Human angiopoietin-2 (ANG-2) with leader and His-tag

<400> 106

```
Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala
1               5                  10                  15

Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
            20                  25                  30

Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro
        35                  40                  45

Glu Met Asp Asn Cys Arg Ser Ser Ser Ser Pro Tyr Val Ser Asn Ala
    50                  55                  60

Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Asp Ser Val Gln Arg Leu
65                  70                  75                  80

Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys
                85                  90                  95

Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Val Glu Ile
            100                 105                 110

Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile Glu Ile Gly
        115                 120                 125

Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
    130                 135                 140

Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu
145                 150                 155                 160

Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln Ile Leu Asp
                165                 170                 175
```

```
Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser Phe Leu Glu
            180                 185                 190

Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln Leu Gln Ser
        195                 200                 205

Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser Lys Gln Asn
    210                 215                 220

Ser Ile Ile Glu Glu Leu Glu Lys Lys Ile Val Thr Ala Thr Val Asn
225                 230                 235                 240

Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu Thr Val Asn
                245                 250                 255

Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Pro Thr
            260                 265                 270

Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Glu Val Phe
        275                 280                 285

Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe Pro Asn
    290                 295                 300

Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly Gly Gly
305                 310                 315                 320

Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp Phe Gln
                325                 330                 335

Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu
            340                 345                 350

Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg
        355                 360                 365

Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr
    370                 375                 380

Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg
385                 390                 395                 400

Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile
```

405 410 415

Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys
420 425 430

Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp
435 440 445

Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln
450 455 460

Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser
465 470 475 480

Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe
485 490 495

Ser Gly His His His His His His
500

<210> 107
<211> 506
<212> PRT
<213> Artificial

<220>
<223> Human angiopoietin-1 (ANG-1) with leader and His-tag

<400> 107

Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His
1 5 10 15

Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg
20 25 30

Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
35 40 45

Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
50 55 60

Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser
65 70 75 80

```
Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp
                85                  90                  95

Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met
            100                 105                 110

Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu
        115                 120                 125

Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys
    130                 135                 140

Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu
145                 150                 155                 160

Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln
                165                 170                 175

Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser
            180                 185                 190

Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu
        195                 200                 205

Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr
    210                 215                 220

Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala
225                 230                 235                 240

Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp
                245                 250                 255

Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu
            260                 265                 270

Lys Gly Gly Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp
        275                 280                 285

Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile
    290                 295                 300

Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn
```

```
305                 310                 315                 320

Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp
                325                 330                 335

Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser
            340                 345                 350

Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln
        355                 360                 365

Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg
    370                 375                 380

Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn
385                 390                 395                 400

Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser
                405                 410                 415

Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn
            420                 425                 430

Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp
        435                 440                 445

Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala
    450                 455                 460

Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys
465                 470                 475                 480

Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu
                485                 490                 495

Asp Phe Ser Gly His His His His His His
            500                 505
```

<210> 108
<211> 1124
<212> PRT
<213> Homo sapiens

<400> 108

```
Met Asp Ser Leu Ala Ser Leu Val Leu Cys Gly Val Ser Leu Leu Leu
1               5                  10                  15

Ser Gly Thr Val Glu Gly Ala Met Asp Leu Ile Leu Ile Asn Ser Leu
            20                  25                  30

Pro Leu Val Ser Asp Ala Glu Thr Ser Leu Thr Cys Ile Ala Ser Gly
        35                  40                  45

Trp Arg Pro His Glu Pro Ile Thr Ile Gly Arg Asp Phe Glu Ala Leu
    50                  55                  60

Met Asn Gln His Gln Asp Pro Leu Glu Val Thr Gln Asp Val Thr Arg
65                  70                  75                  80

Glu Trp Ala Lys Lys Val Val Trp Lys Arg Glu Lys Ala Ser Lys Ile
                85                  90                  95

Asn Gly Ala Tyr Phe Cys Glu Gly Arg Val Arg Gly Glu Ala Ile Arg
            100                 105                 110

Ile Arg Thr Met Lys Met Arg Gln Gln Ala Ser Phe Leu Pro Ala Thr
        115                 120                 125

Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val Asn Ile Ser Phe Lys
    130                 135                 140

Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser
145                 150                 155                 160

Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val
                165                 170                 175

His Leu Pro His Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg
            180                 185                 190

Tyr Ile Gly Gly Asn Leu Phe Thr Ser Ala Phe Thr Arg Leu Ile Val
        195                 200                 205

Arg Arg Cys Glu Ala Gln Lys Trp Gly Pro Glu Cys Asn His Leu Cys
    210                 215                 220
```

```
Thr Ala Cys Met Asn Asn Gly Val Cys His Glu Asp Thr Gly Glu Cys
225                 230                 235                 240

Ile Cys Pro Pro Gly Phe Met Gly Arg Thr Cys Glu Lys Ala Cys Glu
                245                 250                 255

Leu His Thr Phe Gly Arg Thr Cys Lys Glu Arg Cys Ser Gly Gln Glu
            260                 265                 270

Gly Cys Lys Ser Tyr Val Phe Cys Leu Pro Asp Pro Tyr Gly Cys Ser
        275                 280                 285

Cys Ala Thr Gly Trp Lys Gly Leu Gln Cys Asn Glu Ala Cys His Pro
    290                 295                 300

Gly Phe Tyr Gly Pro Asp Cys Lys Leu Arg Cys Ser Cys Asn Asn Gly
305                 310                 315                 320

Glu Met Cys Asp Arg Phe Gln Gly Cys Leu Cys Ser Pro Gly Trp Gln
                325                 330                 335

Gly Leu Gln Cys Glu Arg Glu Gly Ile Pro Arg Met Thr Pro Lys Ile
            340                 345                 350

Val Asp Leu Pro Asp His Ile Glu Val Asn Ser Gly Lys Phe Asn Pro
        355                 360                 365

Ile Cys Lys Ala Ser Gly Trp Pro Leu Pro Thr Asn Glu Glu Met Thr
    370                 375                 380

Leu Val Lys Pro Asp Gly Thr Val Leu His Pro Lys Asp Phe Asn His
385                 390                 395                 400

Thr Asp His Phe Ser Val Ala Ile Phe Thr Ile His Arg Ile Leu Pro
                405                 410                 415

Pro Asp Ser Gly Val Trp Val Cys Ser Val Asn Thr Val Ala Gly Met
            420                 425                 430

Val Glu Lys Pro Phe Asn Ile Ser Val Lys Val Leu Pro Lys Pro Leu
        435                 440                 445

Asn Ala Pro Asn Val Ile Asp Thr Gly His Asn Phe Ala Val Ile Asn
```

```
     450                 455                 460

Ile Ser Ser Glu Pro Tyr Phe Gly Asp Gly Pro Ile Lys Ser Lys Lys
465                 470                 475                 480

Leu Leu Tyr Lys Pro Val Asn His Tyr Glu Ala Trp Gln His Ile Gln
                485                 490                 495

Val Thr Asn Glu Ile Val Thr Leu Asn Tyr Leu Glu Pro Arg Thr Glu
            500                 505                 510

Tyr Glu Leu Cys Val Gln Leu Val Arg Arg Gly Glu Gly Gly Glu Gly
        515                 520                 525

His Pro Gly Pro Val Arg Arg Phe Thr Thr Ala Ser Ile Gly Leu Pro
    530                 535                 540

Pro Pro Arg Gly Leu Asn Leu Leu Pro Lys Ser Gln Thr Thr Leu Asn
545                 550                 555                 560

Leu Thr Trp Gln Pro Ile Phe Pro Ser Ser Glu Asp Asp Phe Tyr Val
                565                 570                 575

Glu Val Glu Arg Arg Ser Val Gln Lys Ser Asp Gln Gln Asn Ile Lys
            580                 585                 590

Val Pro Gly Asn Leu Thr Ser Val Leu Leu Asn Asn Leu His Pro Arg
        595                 600                 605

Glu Gln Tyr Val Val Arg Ala Arg Val Asn Thr Lys Ala Gln Gly Glu
    610                 615                 620

Trp Ser Glu Asp Leu Thr Ala Trp Thr Leu Ser Asp Ile Leu Pro Pro
625                 630                 635                 640

Gln Pro Glu Asn Ile Lys Ile Ser Asn Ile Thr His Ser Ser Ala Val
                645                 650                 655

Ile Ser Trp Thr Ile Leu Asp Gly Tyr Ser Ile Ser Ser Ile Thr Ile
            660                 665                 670

Arg Tyr Lys Val Gln Gly Lys Asn Glu Asp Gln His Val Asp Val Lys
        675                 680                 685
```

```
Ile Lys Asn Ala Thr Ile Thr Gln Tyr Gln Leu Lys Gly Leu Glu Pro
    690                 695                 700

Glu Thr Ala Tyr Gln Val Asp Ile Phe Ala Glu Asn Asn Ile Gly Ser
705                 710                 715                 720

Ser Asn Pro Ala Phe Ser His Glu Leu Val Thr Leu Pro Glu Ser Gln
                725                 730                 735

Ala Pro Ala Asp Leu Gly Gly Gly Lys Met Leu Leu Ile Ala Ile Leu
            740                 745                 750

Gly Ser Ala Gly Met Thr Cys Leu Thr Val Leu Leu Ala Phe Leu Ile
        755                 760                 765

Ile Leu Gln Leu Lys Arg Ala Asn Val Gln Arg Arg Met Ala Gln Ala
    770                 775                 780

Phe Gln Asn Val Arg Glu Glu Pro Ala Val Gln Phe Asn Ser Gly Thr
785                 790                 795                 800

Leu Ala Leu Asn Arg Lys Val Lys Asn Asn Pro Asp Pro Thr Ile Tyr
                805                 810                 815

Pro Val Leu Asp Trp Asn Asp Ile Lys Phe Gln Asp Val Ile Gly Glu
            820                 825                 830

Gly Asn Phe Gly Gln Val Leu Lys Ala Arg Ile Lys Lys Asp Gly Leu
        835                 840                 845

Arg Met Asp Ala Ala Ile Lys Arg Met Lys Glu Tyr Ala Ser Lys Asp
    850                 855                 860

Asp His Arg Asp Phe Ala Gly Glu Leu Glu Val Leu Cys Lys Leu Gly
865                 870                 875                 880

His His Pro Asn Ile Ile Asn Leu Leu Gly Ala Cys Glu His Arg Gly
                885                 890                 895

Tyr Leu Tyr Leu Ala Ile Glu Tyr Ala Pro His Gly Asn Leu Leu Asp
            900                 905                 910
```

```
Phe Leu Arg Lys Ser Arg Val Leu Glu Thr Asp Pro Ala Phe Ala Ile
        915                 920                 925

Ala Asn Ser Thr Ala Ser Thr Leu Ser Ser Gln Gln Leu Leu His Phe
    930                 935                 940

Ala Ala Asp Val Ala Arg Gly Met Asp Tyr Leu Ser Gln Lys Gln Phe
945                 950                 955                 960

Ile His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Gly Glu Asn Tyr
                965                 970                 975

Val Ala Lys Ile Ala Asp Phe Gly Leu Ser Arg Gly Gln Glu Val Tyr
            980                 985                 990

Val Lys Lys Thr Met Gly Arg Leu  Pro Val Arg Trp Met  Ala Ile Glu
        995                 1000                1005

Ser Leu  Asn Tyr Ser Val Tyr  Thr Thr Asn Ser Asp  Val Trp Ser
    1010                1015                1020

Tyr Gly  Val Leu Leu Trp Glu  Ile Val Ser Leu Gly  Gly Thr Pro
    1025                1030                1035

Tyr Cys  Gly Met Thr Cys Ala  Glu Leu Tyr Glu Lys  Leu Pro Gln
    1040                1045                1050

Gly Tyr  Arg Leu Glu Lys Pro  Leu Asn Cys Asp Asp  Glu Val Tyr
    1055                1060                1065

Asp Leu  Met Arg Gln Cys Trp  Arg Glu Lys Pro Tyr  Glu Arg Pro
    1070                1075                1080

Ser Phe  Ala Gln Ile Leu Val  Ser Leu Asn Arg Met  Leu Glu Glu
    1085                1090                1095

Arg Lys  Thr Tyr Val Asn Thr  Thr Leu Tyr Glu Lys  Phe Thr Tyr
    1100                1105                1110

Ala Gly  Ile Asp Cys Ser Ala  Glu Glu Ala Ala
    1115                1120
```

<210> 109
<211> 946
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 1 of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFAb-Avastin-

LC06-2620

<400> 109

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190
```

```
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415
```

```
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln
    450                 455                 460

Pro Gly Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln Thr
465                 470                 475                 480

Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val His
                485                 490                 495

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr Asp
            500                 505                 510

Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser Asn
        515                 520                 525

Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly Asp
    530                 535                 540

Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His Tyr
545                 550                 555                 560

Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Arg Thr Val Ala Ala
                565                 570                 575

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
            580                 585                 590

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
        595                 600                 605

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
    610                 615                 620

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
625                 630                 635                 640
```

```
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
                645                 650                 655

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
            660                 665                 670

Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        675                 680                 685

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    690                 695                 700

Gly Gly Gly Ser Gly Gly Gln Val Gln Leu Val Glu Ser Gly Ala Glu
705                 710                 715                 720

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
                725                 730                 735

Tyr Thr Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly
            740                 745                 750

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
        755                 760                 765

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
    770                 775                 780

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
785                 790                 795                 800

Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp
                805                 810                 815

Ser Ser Gly Tyr Tyr Tyr Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly
            820                 825                 830

Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        835                 840                 845

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    850                 855                 860

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
```

865 870 875 880

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
885 890 895

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
900 905 910

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
915 920 925

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
930 935 940

Thr His
945

<210> 110
<211> 214
<212> PRT
<213> Artificial

<220>
<223> Light chain of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFAb-Avastin-LC06-2620

<400> 110

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
35 40 45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
85 90 95

```
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys
    210
```

<210> 111
<211> 956
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 1 of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFab-Avastin-Ang2i-LC06-2640

<400> 111

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
```

```
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285
```

```
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    450                 455                 460

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Pro Gly Leu Thr Gln Pro
465                 470                 475                 480

Pro Ser Val Ser Val Ala Pro Gly Gln Thr Ala Arg Ile Thr Cys Gly
                485                 490                 495

Gly Asn Asn Ile Gly Ser Lys Ser Val His Trp Tyr Gln Gln Lys Pro
            500                 505                 510
```

```
Gly Gln Ala Pro Val Leu Val Val Tyr Asp Asp Ser Asp Arg Pro Ser
        515                 520                 525

Gly Ile Pro Glu Arg Phe Ser Gly Ser Asn Ser Gly Asn Thr Ala Thr
    530                 535                 540

Leu Thr Ile Ser Arg Val Glu Ala Gly Asp Glu Ala Asp Tyr Tyr Cys
545                 550                 555                 560

Gln Val Trp Asp Ser Ser Ser Asp His Tyr Val Phe Gly Thr Gly Thr
                565                 570                 575

Lys Val Thr Val Leu Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
            580                 585                 590

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
        595                 600                 605

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
    610                 615                 620

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
625                 630                 635                 640

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
                645                 650                 655

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
            660                 665                 670

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
        675                 680                 685

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    690                 695                 700

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
705                 710                 715                 720

Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
                725                 730                 735
```

```
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            740                 745                 750

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        755                 760                 765

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    770                 775                 780

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
785                 790                 795                 800

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                805                 810                 815

Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            820                 825                 830

Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        835                 840                 845

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
    850                 855                 860

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
865                 870                 875                 880

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
                885                 890                 895

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
            900                 905                 910

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
        915                 920                 925

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
    930                 935                 940

Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
945                 950                 955
```

<210> 112
<211> 214
<212> PRT
<213> Artificial

<220>

<223> Light chain of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFab-Avastin-Ang2i-LC06-2640

<400> 112

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys 210
```

<210> 113
<211> 956
<212> PRT

<213> Artificial

<220>
<223> Heavy chain 1 of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFab-Avastin-Ang2i-LC06-2641

<400> 113

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
```

```
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380
```

```
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    450                 455                 460

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Pro Gly Leu Thr Gln Pro
465                 470                 475                 480

Pro Ser Val Ser Val Ala Pro Gly Gln Thr Ala Arg Ile Thr Cys Gly
                485                 490                 495

Gly Asn Asn Ile Gly Ser Lys Ser Val His Trp Tyr Gln Gln Lys Pro
            500                 505                 510

Gly Gln Ala Pro Val Leu Val Val Tyr Asp Asp Ser Asp Arg Pro Ser
        515                 520                 525

Gly Ile Pro Glu Arg Phe Ser Gly Ser Asn Ser Gly Asn Thr Ala Thr
    530                 535                 540

Leu Thr Ile Ser Arg Val Glu Ala Gly Asp Glu Ala Asp Tyr Tyr Cys
545                 550                 555                 560

Gln Val Trp Asp Ser Ser Ser Asp His Tyr Val Phe Gly Cys Gly Thr
                565                 570                 575

Lys Val Thr Val Leu Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
            580                 585                 590

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
        595                 600                 605
```

```
Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
    610                 615                 620

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
625                 630                 635                 640

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
                645                 650                 655

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
            660                 665                 670

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
        675                 680                 685

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    690                 695                 700

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
705                 710                 715                 720

Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
                725                 730                 735

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            740                 745                 750

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Cys Leu Glu Trp Met
        755                 760                 765

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    770                 775                 780

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
785                 790                 795                 800

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                805                 810                 815

Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            820                 825                 830
```

```
Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        835                 840                 845

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
    850                 855                 860

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
865                 870                 875                 880

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
                885                 890                 895

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
            900                 905                 910

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
        915                 920                 925

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
    930                 935                 940

Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
945                 950                 955
```

<210> 114
<211> 214
<212> PRT
<213> Artificial

<220>
<223> Light chain of bispecific, tetravalent single chain Fab <VEGF-ANG-2> antibody molecule scFab-Avastin-Ang2i-LC06-2641

<400> 114

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
```

```
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys
    210
```

<210> 115
<211> 946
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 1 of bispecific, trivalent single chain Fab <VEGF-ANG-2> antibody molecule Avastin-LC06-KiH-C-scFab

<400> 115

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
```

```
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

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

245 250 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
290 295 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305 310 315 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
325 330 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
340 345 350

Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln
355 360 365

Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
370 375 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385 390 395 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
405 410 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
420 425 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
435 440 445

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln
450 455 460

Pro Gly Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln Thr
465 470 475 480

```
Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val His
                485                 490                 495

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr Asp
            500                 505                 510

Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser Asn
        515                 520                 525

Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly Asp
    530                 535                 540

Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His Tyr
545                 550                 555                 560

Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Arg Thr Val Ala Ala
                565                 570                 575

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
            580                 585                 590

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
        595                 600                 605

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
    610                 615                 620

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
625                 630                 635                 640

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
                645                 650                 655

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
            660                 665                 670

Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        675                 680                 685

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    690                 695                 700
```

```
Gly Gly Gly Ser Gly Gly Gln Val Gln Leu Val Glu Ser Gly Ala Glu
705                 710                 715                 720

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
                725                 730                 735

Tyr Thr Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly
            740                 745                 750

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
        755                 760                 765

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
    770                 775                 780

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
785                 790                 795                 800

Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp
                805                 810                 815

Ser Ser Gly Tyr Tyr Tyr Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly
            820                 825                 830

Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        835                 840                 845

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    850                 855                 860

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
865                 870                 875                 880

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                885                 890                 895

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            900                 905                 910

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        915                 920                 925

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
    930                 935                 940

Thr His
945
```

<210> 116
<211> 453
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 2 of bispecific, trivalent single chain Fab <VEGF-ANG-2> antibody molecule Avastin-LC06-KiH-C-scFab

<400> 116

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160
```

```
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380
```

```
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu
                405                 410                 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445

Leu Ser Pro Gly Lys
    450
```

<210> 117
<211> 214
<212> PRT
<213> Artificial

<220>
<223> Light chain of bispecific, trivalent single chain Fab <VEGF-ANG-2> antibody molecule Avastin-LC06-KiH-C-scFab

<400> 117

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
```

100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 118
<211> 698
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 1 of bispecific, trivalent <VEGF-ANG-2> antibody molecule Avastin-LC06-C-Fab-6CSS

<400> 118

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
50 55 60

```
Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285
```

```
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    450                 455                 460

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
465                 470                 475                 480

Gly Gly Ser Gln Pro Gly Leu Thr Gln Pro Pro Ser Val Ser Val Ala
                485                 490                 495

Pro Gly Gln Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser
            500                 505                 510

Lys Ser Val His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu
```

```
                515                 520                 525

Val Val Tyr Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe
    530                 535                 540

Ser Gly Ser Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val
545                 550                 555                 560

Glu Ala Gly Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser
                565                 570                 575

Ser Asp His Tyr Val Phe Gly Cys Gly Thr Lys Val Thr Val Leu Arg
            580                 585                 590

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
        595                 600                 605

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
    610                 615                 620

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
625                 630                 635                 640

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
                645                 650                 655

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
            660                 665                 670

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
        675                 680                 685

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    690                 695
```

<210> 119
<211> 719
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 2 of bispecific, trivalent <VEGF-ANG-2> antibody molecule Avastin-LC06-C-Fab-6CSS

<400> 119

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220
```

```
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350

Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365

Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385                 390                 395                 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu
                405                 410                 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
```

```
    450                 455                 460

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
465                 470                 475                 480

Gly Gly Ser Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys
                485                 490                 495

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
            500                 505                 510

Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Cys Leu
        515                 520                 525

Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala
    530                 535                 540

Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser
545                 550                 555                 560

Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val
                565                 570                 575

Tyr Tyr Cys Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly
            580                 585                 590

Tyr Tyr Tyr Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val
        595                 600                 605

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
    610                 615                 620

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
625                 630                 635                 640

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
                645                 650                 655

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
            660                 665                 670

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
        675                 680                 685
```

```
Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
690                 695                 700

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
705                 710                 715
```

<210> 120
<211> 214
<212> PRT
<213> Artificial

<220>
<223> Light chain of bispecific, trivalent <VEGF-ANG-2> antibody molecule Avastin-LC06-C-Fab-6CSS

<400> 120

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140
```

```
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys
    210
```

<210> 121
<211> 459
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 1 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-CH1-CL

<400> 121

```
Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110
```

```
Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
    130                 135                 140

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
145                 150                 155                 160

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
                165                 170                 175

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
            180                 185                 190

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
        195                 200                 205

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
    210                 215                 220

Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
225                 230                 235                 240

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
                245                 250                 255

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
            260                 265                 270

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
        275                 280                 285

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
    290                 295                 300

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
305                 310                 315                 320

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
                325                 330                 335
```

```
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
            340                 345                 350

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp
        355                 360                 365

Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe
    370                 375                 380

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
385                 390                 395                 400

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
                405                 410                 415

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
            420                 425                 430

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
        435                 440                 445

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455
```

<210> 122
<211> 457
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-CH1-CL

<400> 122

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
```

```
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Val Ala Ala
        115                 120                 125

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
    130                 135                 140

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
145                 150                 155                 160

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
                165                 170                 175

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
            180                 185                 190

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
        195                 200                 205

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
    210                 215                 220

Phe Asn Arg Gly Glu Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
225                 230                 235                 240

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
                245                 250                 255

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            260                 265                 270

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
        275                 280                 285
```

```
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    290                 295                 300

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
305                 310                 315                 320

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                325                 330                 335

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
            340                 345                 350

Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu
        355                 360                 365

Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro
    370                 375                 380

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
385                 390                 395                 400

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                405                 410                 415

Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
            420                 425                 430

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
        435                 440                 445

Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455
```

<210> 123
<211> 215
<212> PRT
<213> Artificial

<220>
<223> Light chain 1 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-CH1-CL

<400> 123

```
Gln Pro Gly Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1               5                  10                  15

Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45

Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80

Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95

Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Arg Thr Val Ala
            100                 105                 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 124
<211> 212
<212> PRT
<213> Artificial

<220>

<223> Light chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-CH1-CL

<400> 124

```
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95

Thr Phe Gly Cys Gly Thr Lys Val Glu Ile Lys Ser Ser Ala Ser Thr
            100                 105                 110

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
        115                 120                 125

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
    130                 135                 140

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
145                 150                 155                 160

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
                165                 170                 175

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
            180                 185                 190

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
        195                 200                 205

Pro Lys Ser Cys
    210
```

<210> 125

<211> 459
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 1 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-VH-VL

<400> 125

```
Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110

Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
    130                 135                 140

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
```

```
145                 150                 155                 160

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
                165                 170                 175

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
            180                 185                 190

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
        195                 200                 205

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
    210                 215                 220

Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
225                 230                 235                 240

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
                245                 250                 255

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
            260                 265                 270

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
        275                 280                 285

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
    290                 295                 300

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
305                 310                 315                 320

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
                325                 330                 335

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
            340                 345                 350

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp
        355                 360                 365

Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe
    370                 375                 380
```

```
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
385                 390                 395                 400

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
                405                 410                 415

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
            420                 425                 430

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
        435                 440                 445

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455
```

<210> 126
<211> 439
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-VH-VL

<400> 126

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95
```

```
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Ser Ser Ala Ser Thr
            100                 105                 110

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
        115                 120                 125

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
    130                 135                 140

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
145                 150                 155                 160

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
                165                 170                 175

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
            180                 185                 190

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
        195                 200                 205

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
    210                 215                 220

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
225                 230                 235                 240

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
                245                 250                 255

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
            260                 265                 270

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
        275                 280                 285

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
    290                 295                 300

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
305                 310                 315                 320

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
```

325 330 335

Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
340 345 350

Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp
355 360 365

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
370 375 380

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser
385 390 395 400

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
405 410 415

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
420 425 430

Leu Ser Leu Ser Pro Gly Lys
435

<210> 127
<211> 215
<212> PRT
<213> Artificial

<220>
<223> Light chain 1 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-VH-VL

<400> 127

Gln Pro Gly Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1 5 10 15

Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
35 40 45

Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

```
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80

Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95

Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Arg Thr Val Ala
            100                 105                 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 128
<211> 230
<212> PRT
<213> Artificial

<220>
<223> Light chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-VH-VL

<400> 128

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
```

```
                20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Val Ala Ala
        115                 120                 125

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
    130                 135                 140

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
145                 150                 155                 160

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
                165                 170                 175

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
            180                 185                 190

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
        195                 200                 205

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
    210                 215                 220

Phe Asn Arg Gly Glu Cys
225                 230
```

<210> 129
<211> 459
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 1 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-

VH-VL-SS

<400> 129

```
Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Cys Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
            100                 105                 110

Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
        115                 120                 125

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
    130                 135                 140

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
145                 150                 155                 160

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
                165                 170                 175

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
            180                 185                 190
```

```
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
        195                 200                 205

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
    210                 215                 220

Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
225                 230                 235                 240

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
                245                 250                 255

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
            260                 265                 270

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
        275                 280                 285

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
    290                 295                 300

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
305                 310                 315                 320

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
                325                 330                 335

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
            340                 345                 350

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp
        355                 360                 365

Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe
    370                 375                 380

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
385                 390                 395                 400

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
                405                 410                 415

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
```

420 425 430

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
435 440 445

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 130
<211> 439
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-VH-VL-SS

<400> 130

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
35 40 45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Ser Ser Ala Ser Thr
100 105 110

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
115 120 125

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
130 135 140

```
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
145                 150                 155                 160

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
                165                 170                 175

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
            180                 185                 190

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
        195                 200                 205

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
    210                 215                 220

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
225                 230                 235                 240

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
                245                 250                 255

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
            260                 265                 270

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
        275                 280                 285

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
    290                 295                 300

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
305                 310                 315                 320

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
                325                 330                 335

Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
            340                 345                 350

Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp
        355                 360                 365
```

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
370 375 380

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser
385 390 395 400

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
405 410 415

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
420 425 430

Leu Ser Leu Ser Pro Gly Lys
435

<210> 131
<211> 215
<212> PRT
<213> Artificial

<220>
<223> Light chain 1 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-VH-VL-SS

<400> 131

Gln Pro Gly Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1 5 10 15

Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
35 40 45

Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
85 90 95

Tyr Val Phe Gly Cys Gly Thr Lys Val Thr Val Leu Arg Thr Val Ala
100 105 110

```
Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 132
<211> 230
<212> PRT
<213> Artificial

<220>
<223> Light chain 2 of bispecific, bivalent domain exchanged <VEGF-ANG-2> antibody molecule Avastin-LC06-VH-VL-SS

<400> 132

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
    50                  55                  60
```

```
Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser His Trp Tyr Phe Asp Val
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Val Ala Ala
        115                 120                 125

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
    130                 135                 140

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
145                 150                 155                 160

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
                165                 170                 175

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
            180                 185                 190

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
        195                 200                 205

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
    210                 215                 220

Phe Asn Arg Gly Glu Cys
225                 230
```

<210> 133
<211> 706
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 1 of bispecific, bivalent ScFab-Fc fusion <VEGF-ANG-2> antibody molecule Avastin-LC06-N-scFab

<400> 133

```
Gln Pro Gly Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1               5                   10                  15
```

```
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45

Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80

Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95

Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Arg Thr Val Ala
            100                 105                 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly
    210                 215                 220

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
225                 230                 235                 240
```

```
Gly Gly Gly Gly Ser Gly Gly Gln Val Gln Leu Val Glu Ser Gly Ala
                245                 250                 255

Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser
            260                 265                 270

Gly Tyr Thr Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro
        275                 280                 285

Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly
    290                 295                 300

Thr Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp
305                 310                 315                 320

Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp
                325                 330                 335

Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr
            340                 345                 350

Asp Ser Ser Gly Tyr Tyr Tyr Pro Gly Ala Phe Asp Ile Trp Gly Gln
        355                 360                 365

Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
    370                 375                 380

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
385                 390                 395                 400

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
                405                 410                 415

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
            420                 425                 430

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
        435                 440                 445

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
    450                 455                 460
```

```
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
465                 470                 475                 480

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
                485                 490                 495

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
            500                 505                 510

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
        515                 520                 525

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
    530                 535                 540

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
545                 550                 555                 560

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
                565                 570                 575

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
            580                 585                 590

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
        595                 600                 605

Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
    610                 615                 620

Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
625                 630                 635                 640

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
                645                 650                 655

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
            660                 665                 670

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
        675                 680                 685

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
```

690 695 700

Gly Lys
705

<210> 134
<211> 699
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 2 of bispecific, bivalent ScFab-Fc fusion <VEGF-ANG-2> antibody molecule Avastin-LC06-N-scFab

<400> 134

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
35 40 45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

```
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    210                 215                 220

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
225                 230                 235                 240

Gly Gly Gly Ser Gly Gly Glu Val Gln Leu Val Glu Ser Gly Gly Gly
                245                 250                 255

Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
            260                 265                 270

Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly
        275                 280                 285

Lys Gly Leu Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro
    290                 295                 300

Thr Tyr Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr
305                 310                 315                 320

Ser Lys Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                325                 330                 335

Thr Ala Val Tyr Tyr Cys Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser
            340                 345                 350

His Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser
        355                 360                 365

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
    370                 375                 380
```

```
Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
385                 390                 395                 400

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
                405                 410                 415

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
            420                 425                 430

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
        435                 440                 445

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
    450                 455                 460

Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
465                 470                 475                 480

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
                485                 490                 495

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
            500                 505                 510

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
        515                 520                 525

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
    530                 535                 540

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
545                 550                 555                 560

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
                565                 570                 575

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
            580                 585                 590

Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp
        595                 600                 605

Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe
```

610 615 620

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
625 630 635 640

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
645 650 655

Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
660 665 670

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
675 680 685

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
690 695

<210> 135
<211> 706
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 1 of bispecific, bivalent ScFab-Fc fusion <VEGF-ANG-2> antibody molecule Avastin-LC06-N-scFabSS

<400> 135

Gln Pro Gly Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1 5 10 15

Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
35 40 45

Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
85 90 95

```
Tyr Val Phe Gly Cys Gly Thr Lys Val Thr Val Leu Arg Thr Val Ala
            100                 105                 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly
    210                 215                 220

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
225                 230                 235                 240

Gly Gly Gly Gly Ser Gly Gly Gln Val Gln Leu Val Glu Ser Gly Ala
                245                 250                 255

Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser
            260                 265                 270

Gly Tyr Thr Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro
        275                 280                 285

Gly Gln Cys Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly
    290                 295                 300

Thr Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp
305                 310                 315                 320
```

```
Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp
                325                 330                 335

Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr
            340                 345                 350

Asp Ser Ser Gly Tyr Tyr Tyr Pro Gly Ala Phe Asp Ile Trp Gly Gln
        355                 360                 365

Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
    370                 375                 380

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
385                 390                 395                 400

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
                405                 410                 415

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
            420                 425                 430

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
        435                 440                 445

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
    450                 455                 460

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
465                 470                 475                 480

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
                485                 490                 495

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
            500                 505                 510

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
        515                 520                 525

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
    530                 535                 540

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
```

545 550 555 560

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
565 570 575

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
580 585 590

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
595 600 605

Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
610 615 620

Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
625 630 635 640

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
645 650 655

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
660 665 670

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
675 680 685

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
690 695 700

Gly Lys
705

<210> 136
<211> 699
<212> PRT
<213> Artificial

<220>
<223> Heavy chain 2 of bispecific, bivalent ScFab-Fc fusion <VEGF-ANG-2> antibody molecule Avastin-LC06-N-scFabSS

<400> 136

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

```
Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95

Thr Phe Gly Cys Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    210                 215                 220

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
225                 230                 235                 240
```

```
Gly Gly Gly Ser Gly Gly Glu Val Gln Leu Val Glu Ser Gly Gly Gly
                245                 250                 255

Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
            260                 265                 270

Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly
        275                 280                 285

Lys Cys Leu Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro
    290                 295                 300

Thr Tyr Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr
305                 310                 315                 320

Ser Lys Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                325                 330                 335

Thr Ala Val Tyr Tyr Cys Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser
            340                 345                 350

His Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser
        355                 360                 365

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
    370                 375                 380

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
385                 390                 395                 400

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
                405                 410                 415

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
            420                 425                 430

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
        435                 440                 445

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
    450                 455                 460

Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
```

```
465                 470                 475                 480

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
                485                 490                 495

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
            500                 505                 510

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
        515                 520                 525

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
    530                 535                 540

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
545                 550                 555                 560

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
                565                 570                 575

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
            580                 585                 590

Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp
        595                 600                 605

Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe
    610                 615                 620

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
625                 630                 635                 640

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
                645                 650                 655

Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
            660                 665                 670

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
        675                 680                 685

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    690                 695
```

Claims

1. A bispecific antibody specifically binding to human vascular endothelial growth factor (VEGF) and human angiopoietin-2 (ANG-2) comprising a first antigen-binding site that specifically binds to human VEGF and a second antigen-binding site that specifically binds to human ANG-2,
wherein

i) said antigen-binding sites are each a pair of an antibody heavy chain variable domain and an antibody light chain variable domain;
ii) said first antigen-binding site specifically binding to VEGF comprises in the heavy chain variable domain a CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO: 2, and a CDR1 region of SEQ ID NO:3, and in the light chain variable domain a CDR3 region of SEQ ID NO: 4, a CDR2 region of SEQ ID NO:5, and a CDR1 region of SEQ ID NO:6;
iii) said second antigen-binding site specifically binding to ANG-2 comprises in the heavy chain variable domain a CDR3 region of SEQ ID NO: 46, a CDR2 region of SEQ ID NO: 47, and a CDR1 region of SEQ ID NO: 48, and in the light chain variable domain a CDR3 region of SEQ ID NO: 49, a CDR2 region of SEQ ID NO: 50, and a CDR1 region of SEQ ID NO: 51; and
iv) said antibody is bivalent.

2. The bispecific antibody according to claim 1, **characterized in that** the ratio of the binding affinities KD of the antigen-binding site specific for VEGF/KD of the antigen-binding site specific for ANG-2 is 1.0- 10.0.

3. The bispecific antibody according to any one of claims 1 to 2, **characterized in that** the second antigen-binding site that specifically binds to human ANG-2 is not specifically binding to human angiopoietin 1 (ANG-1).

4. A pharmaceutical composition comprising an antibody according to any one of claims 1 to 3.

5. The pharmaceutical composition according to claim 4 for use in the treatment of cancer.

6. The pharmaceutical composition according to claim 4 for use in the treatment of vascular diseases.

7. The bispecific antibody according to any one of claims 1 to 3 for use in the treatment of cancer.

8. The bispecific antibody according to any one of claims 1 to 3 for use in the treatment of vascular diseases.

9. A nucleic acid encoding a bispecific antibody according to any one of claims 1 to 3.

10. An expression vector containing the nucleic acid according to claim 10 capable of expressing said nucleic acid in a prokaryotic or eukaryotic host cell.

11. A prokaryotic or eukaryotic host cell comprising a vector according to claim 10.

12. A method for the production of a bispecific antibody according to any one of claims 1 to 3, **characterized by** expressing a nucleic acid according to claim 9 in a prokaryotic or eukaryotic host cell and recovering said bispecific antibody from said cell or the cell culture supernatant.

Patentansprüche

1. Bispezifischer Antikörper, der spezifisch an humanen vaskulären endothelialen Wachstumsfaktor (VEGF) und humanes Angiopoetin-2 (ANG-2) bindet, umfassend eine erste Antigenbindestelle, die spezifisch an humanen VEGF bindet und eine zweite Antigenbindestelle, die spezifisch an humanes ANG-2 bindet, wobei

i) jede einzelne der Antigenbindestellen ein Paar aus einer variablen Domäne einer schweren Kette eines Antikörpers und einer variablen Domäne einer leichten Kette eines Antikörpers ist;
ii) die erste Antigenbindestelle, die spezifisch VEGF bindet, in der variablen Domäne der schweren Kette eine CDR3-Region von SEQ ID NO:1, eine CDR2-Region von SEQ ID NO:2 und eine CDR1-Region von SEQ ID NO:3 und in der variablen Domäne der leichten Kette eine CDR3-Region von SEQ ID NO:4, eine CDR2-Region

von SEQ ID NO:5 und eine CDR1-Region von SEQ ID NO:6 umfasst;
iii) die zweite Antigenbindestelle, die spezifisch ANG-2 bindet, in der variablen Domäne der schweren Kette eine CDR3-Region von SEQ ID NO:46, eine CDR2-Region von SEQ ID NO:47 und eine CDR1-Region von SEQ ID NO:48 und in der variablen Domäne der leichten Kette eine CDR3-Region von SEQ ID NO:49, eine CDR2-Region von SEQ ID NO:50 und eine CDR1- egion von SEQ ID NO:51 umfasst; und
iv) der Antikörper bivalent ist.

2. Bispezifischer Antikörper nach Anspruch 1, **dadurch gekennzeichnet, dass** das Verhältnis der Bindeaffinitäten: KD der Antigenbindestelle, die spezifisch für VEGF ist / KD der Antigenbindestelle, die spezifisch für ANG-2 ist, 1.0-10.0 ist.

3. Bispezifischer Antikörper nach einem der Ansprüche 1 bis 2, **dadurch gekennzeichnet, dass** die zweite Antigenbindestelle, die spezifisch an humanes ANG-2 bindet, nicht spezifisch an humanes Angiopoetin 1 (ANG-1) bindet.

4. Arzneimittel, umfassend einen Antikörper nach einem der Ansprüche 1 bis 3.

5. Arzneimittel nach Anspruch 4 zur Verwendung bei der Behandlung von Krebs.

6. Arzneimittel nach Anspruch 4 zur Verwendung bei der Behandlung vaskulärer Erkrankungen.

7. Bispezifischer Antikörper nach einem der Ansprüche 1 bis 3 zur Verwendung bei der Behandlung von Krebs.

8. Bispezifischer Antikörper nach einem der Ansprüche 1 bis 3 zur Verwendung bei der Behandlung von vaskulären Erkrankungen.

9. Nucleinsäure, die einen bispezifischen Antikörper nach einem der Ansprüche 1 bis 3 codiert.

10. Expressionsvektor enthaltend die Nucleinsäure nach Anspruch 10, der in der Lage ist, die Nucleinsäure in einer prokaryotischen oder eukaryotischen Wirtszelle zu exprimieren.

11. Prokaryotische oder eukaryotische Wirtszelle umfassend einen Vektor nach Anspruch 10.

12. Verfahren zur Herstellung eines bispezifischen Antikörpers nach einem der Ansprüche 1 bis 3, **gekennzeichnet durch** die Expression einer Nucleinsäure nach Anspruch 9 in einer prokaryotischen oder eukaryotischen Wirtszelle und das Gewinnen des bispezifischen Antikörpers von der Zelle oder von dem Zellkulturüberstand.

Revendications

1. Anticorps bispécifique se liant spécifiquement à un facteur de croissance endothélial vasculaire humain (VEGF) et à l'angiopoïétine-2 humaine (ANG-2), comprenant un premier site de liaison d'antigène qui se lie spécifiquement au VEGF humain et un second site de liaison d'antigène qui se lie spécifiquement à la ANG-2 humaine, dans lequel,

i) lesdits sites de liaison d'antigène représentent chacun une paire d'un domaine variable de chaîne lourde d'anticorps et d'un domaine variable de chaîne légère d'anticorps ;
ii) ledit premier site de liaison d'antigène se liant spécifiquement au VEGF comprend dans le domaine variable de chaîne lourde une région CDR3 de la SEQ ID n°: 1, une région CDR2 de la SEQ ID n°: 2 et une région CDR1 de la SEQ ID n°: 3, et dans le domaine variable de chaîne légère une région CDR3 de la SEQ ID n°: 4, une région CDR2 de la SEQ ID n°: 5 et une région CDR1 de la SEQ ID n°: 6 ;
iii) ledit second site de liaison d'antigène se liant spécifiquement à la ANG-2 comprend dans le domaine variable de chaîne lourde une région CDR3 de la SEQ ID n°: 46, une région CDR2 de la SEQ ID n°: 47 et une région CDR1 de la SEQ ID n°: 48, et dans le domaine variable de chaîne légère une région CDR3 de la SEQ ID n°: 49, une région CDR2 de la SEQ ID n°: 50 et une région CDR1 de la SEQ ID n°: 51 ; et
iv) ledit anticorps est bivalent.

2. Anticorps bispécifique suivant la revendication 1, **caractérisé en ce que** le rapport des affinités de liaison KD du site de liaison d'antigène spécifique du VEGF/KD au site de liaison d'antigène spécifique de ANG-2 a une valeur

de 1,0-10,0.

3. Anticorps bispécifique suivant l'une quelconque des revendications 1 et 2, **caractérisé en ce que** le second site de liaison d'antigène qui se lie spécifiquement à la ANG-2 humaine ne se lie pas spécifiquement à l'angiopoïétine 1 humaine (ANG-1).

4. Composition pharmaceutique comprenant un anticorps suivant l'une quelconque des revendications 1 à 3.

5. Composition pharmaceutique suivant la revendication 4, pour une utilisation dans le traitement du cancer.

6. Composition pharmaceutique suivant la revendication 4, pour une utilisation dans le traitement de maladies vasculaires.

7. Anticorps bispécifique suivant l'une quelconque des revendications 1 à 3, pour une utilisation dans le traitement du cancer.

8. Anticorps bispécifique suivant l'une quelconque des revendications 1 à 3, pour une utilisation dans le traitement de maladies vasculaires.

9. Acide nucléique codant pour un anticorps bispécifique suivant l'une quelconque des revendications 1 à 3.

10. Vecteur d'expression contenant l'acide nucléique suivant la revendication 10, apte à l'expression dudit acide nucléique dans une cellule hôte procaryotique ou eucaryotique.

11. Cellule hôte procaryotique ou eucaryotique comprenant un vecteur suivant la revendication 10.

12. Procédé pour la production d'un anticorps bispécifique suivant l'une quelconque des revendications 1 à 3, **caractérisé par** l'expression d'un acide nucléique suivant la revendication 9 dans une cellule hôte procaryotique ou eucaryotique et la récupération dudit anticorps bispécifique à partir de ladite cellule ou du surnageant de culture cellulaire.

Figure 1A

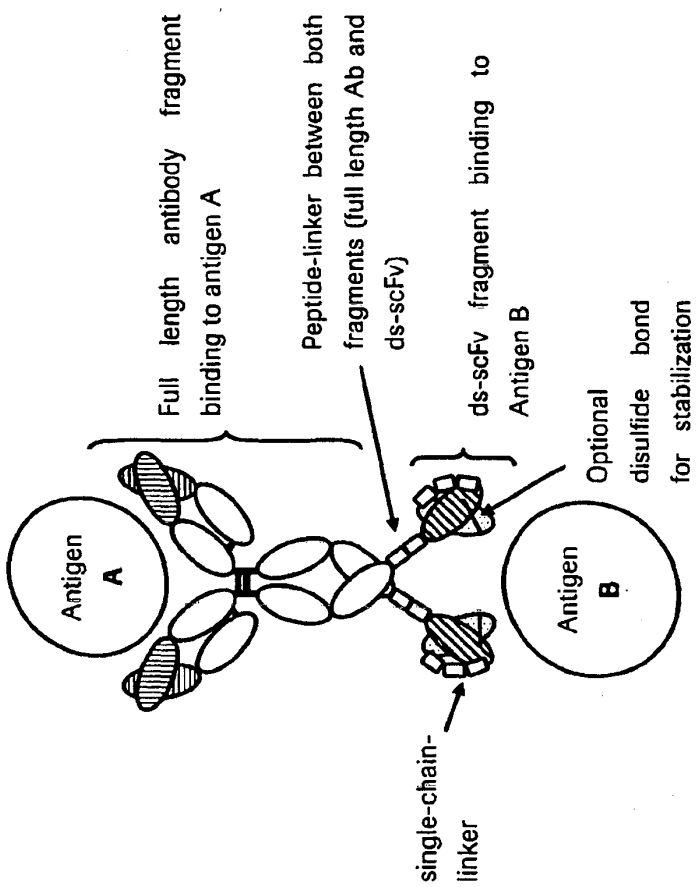

Full length antibody fragment binding to antigen A
Peptide-linker between both fragments (full length Ab and ds-scFv)
ds-scFv fragment binding to Antigen B
Optional disulfide bond for stabilization
Antigen A
Antigen B
single-chain-linker

Figure 1B

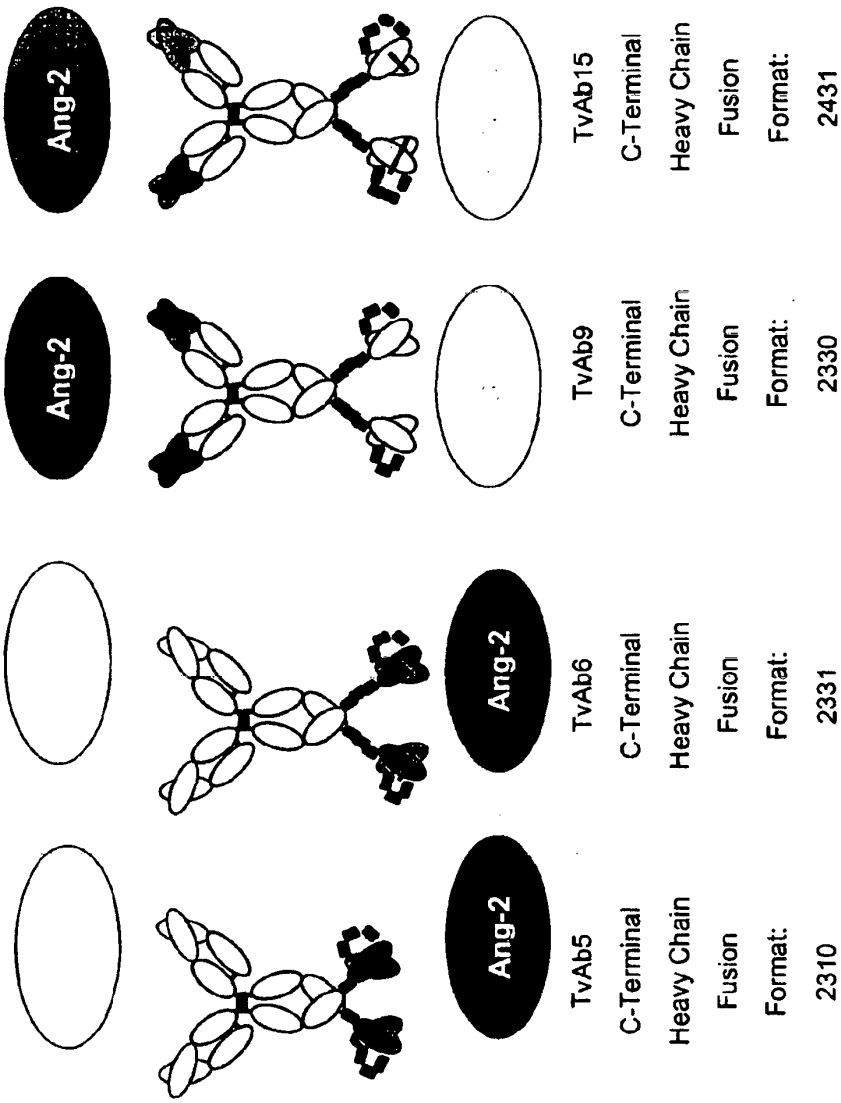

Ang-2
Ang-2
Ang-2
TvAb5
C-Terminal
Heavy Chain
Fusion
Format:
2310
TvAb6
C-Terminal
Heavy Chain
Fusion
Format:
2331
TvAb9
C-Terminal
Heavy Chain
Fusion
Format:
2330
TvAb15
C-Terminal
Heavy Chain
Fusion
Format:
2431

Figure 2A

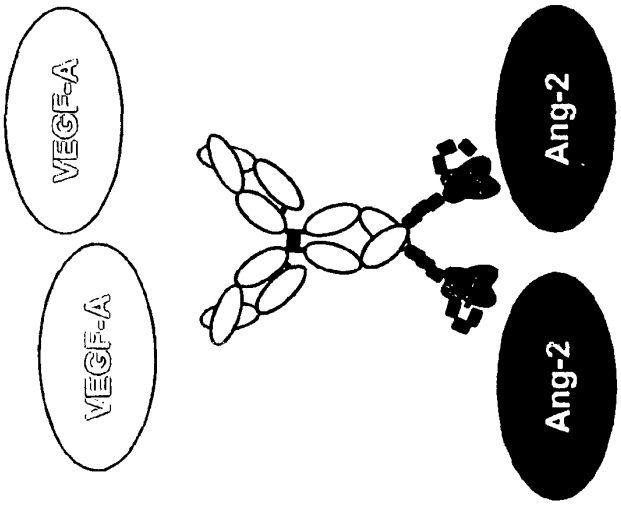


Figure 2 - part one

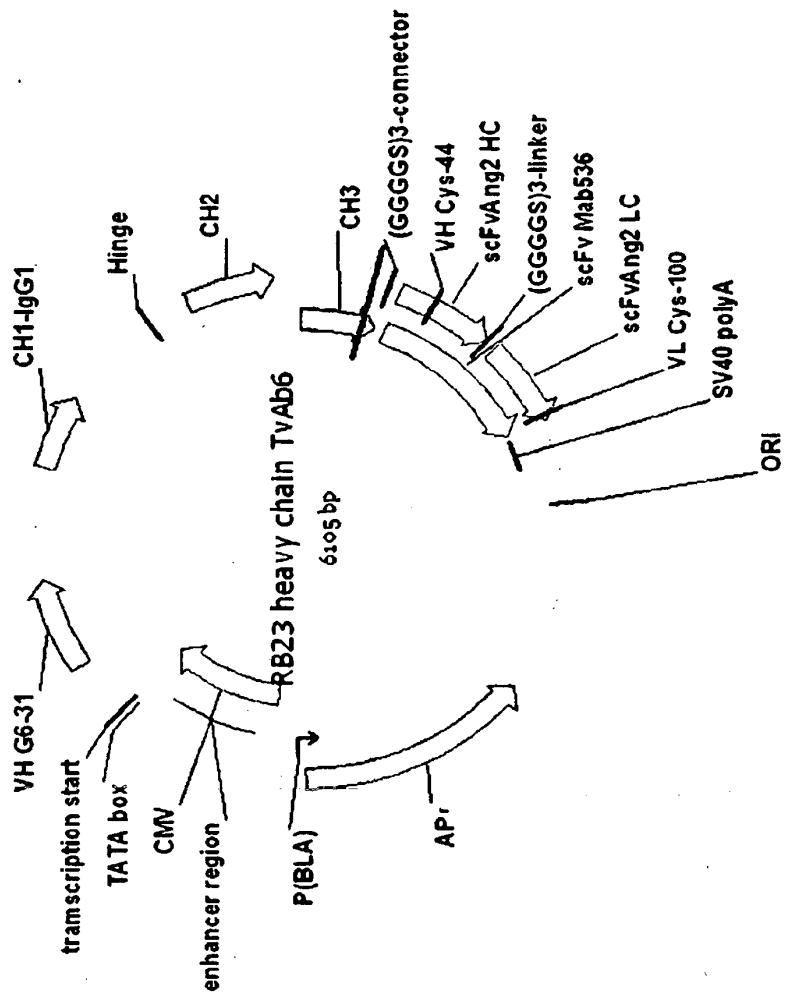


Figure 2b-part two

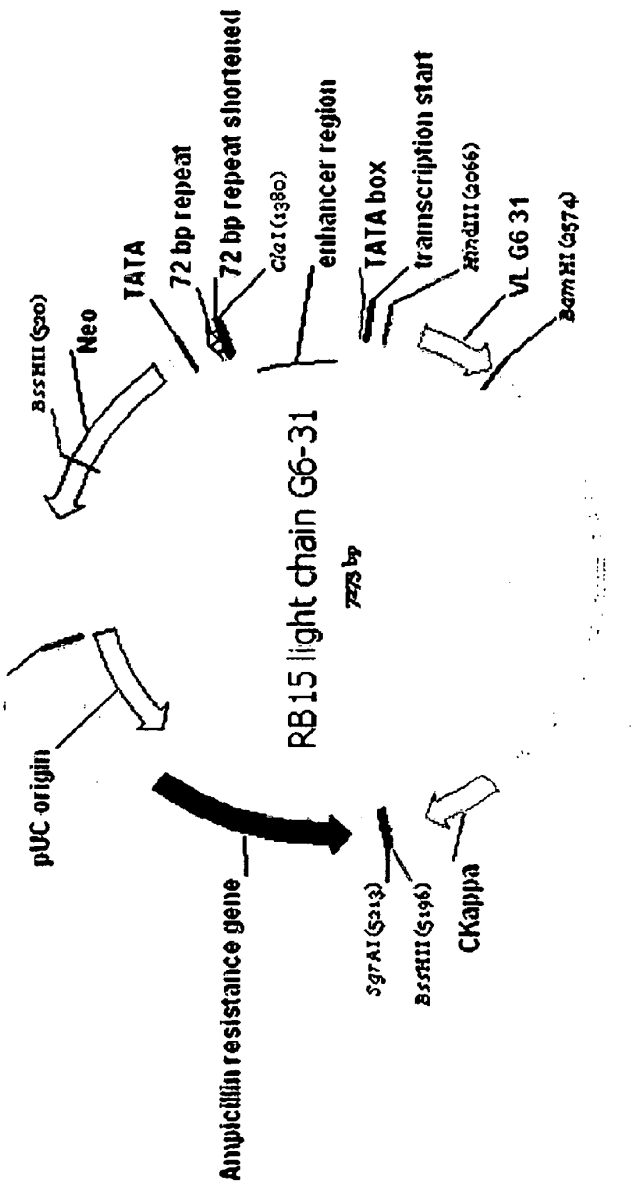


Figure 3

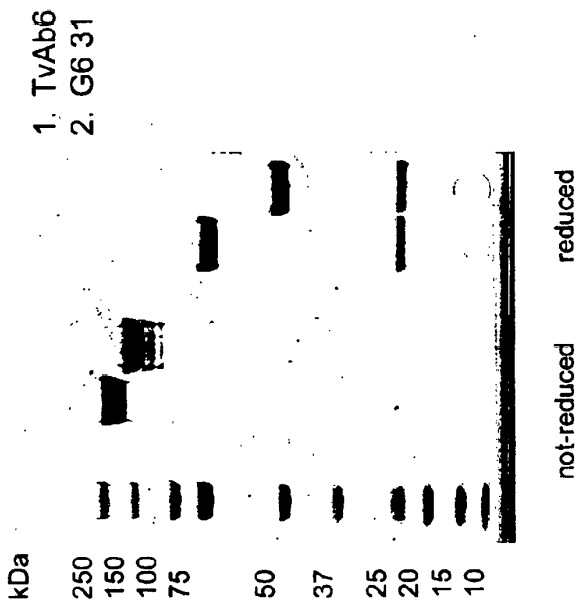

1. TvAb6
2. G6 31
1 2 1 2
kDa
250
150
100
75
50
37
25
20
15
10
not-reduced
reduced

Fig. 4A

1. Protein A Pool

G6-31: 10 % aggregates

TvAb6: 25 % aggregates

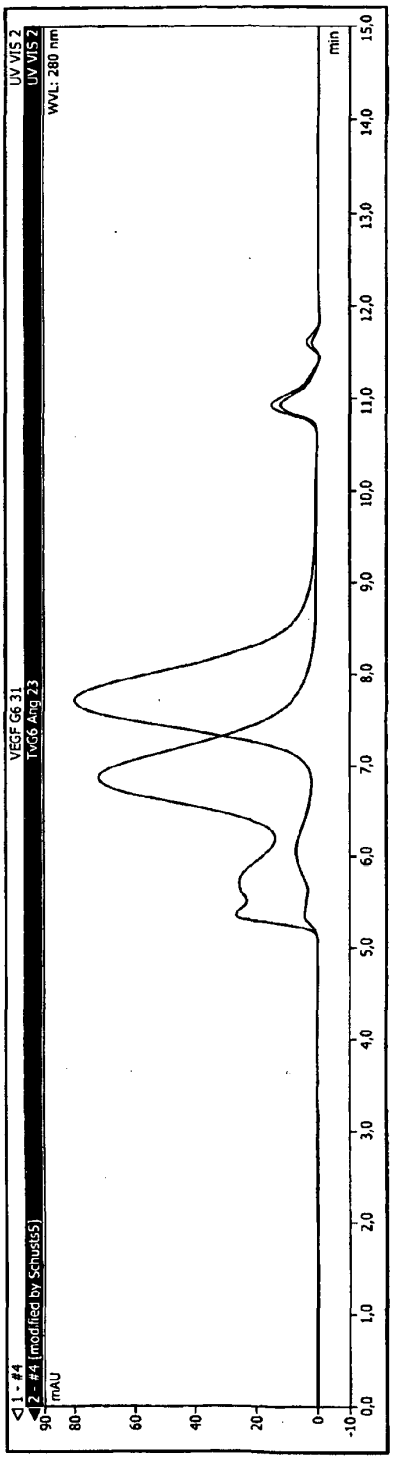


Fig. 4B

2. SEC Pool, after purification

G6-31: > 99 % monomer

TvAb6: > 99 % monomer

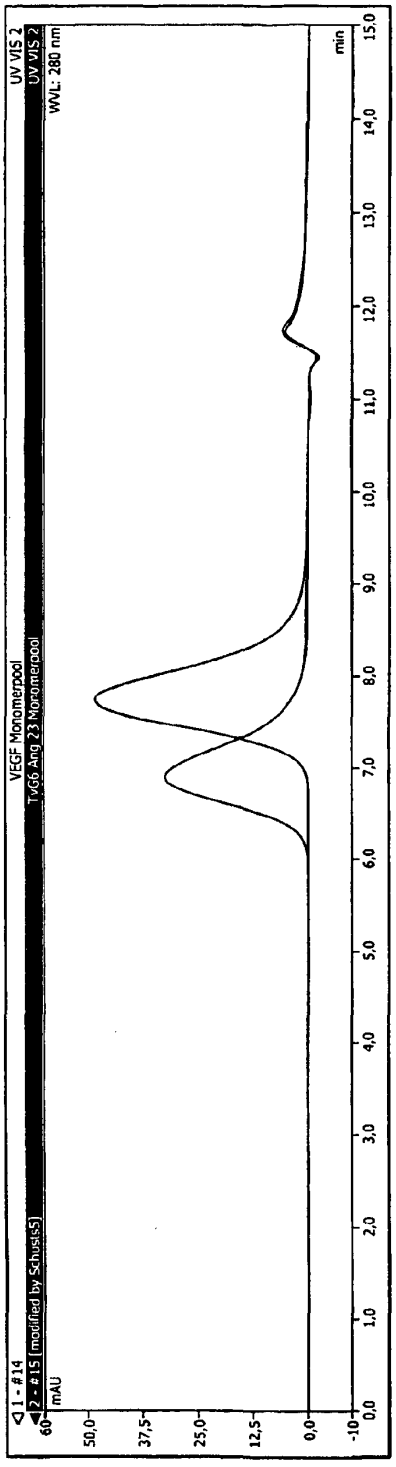


Fig. 4C

3. SEC Pool, after 24 h at 4 °C

G6-31: > 99 % monomer

TvAb6: > 99 % monomer

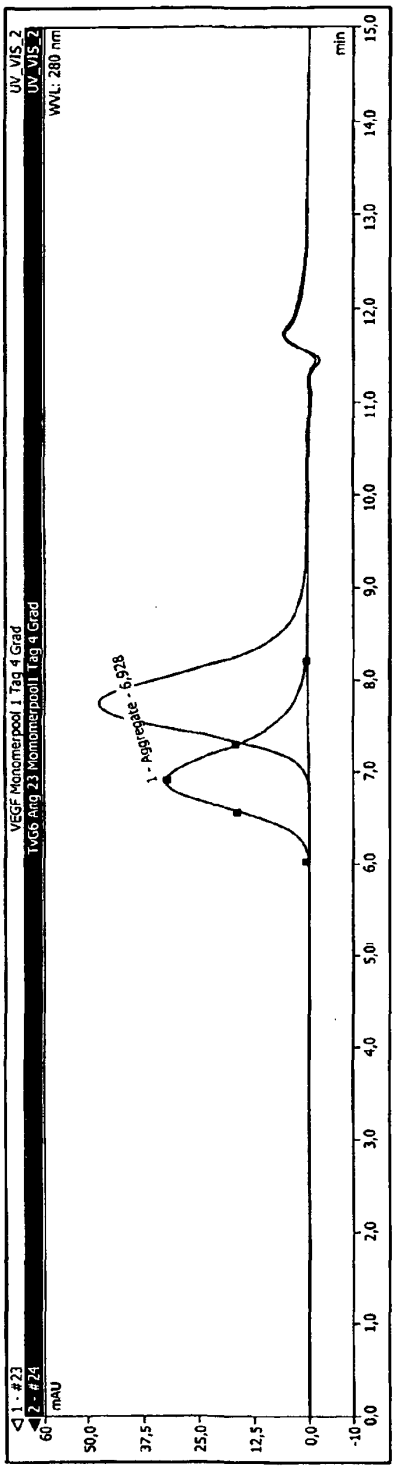


Figure 5 –part one

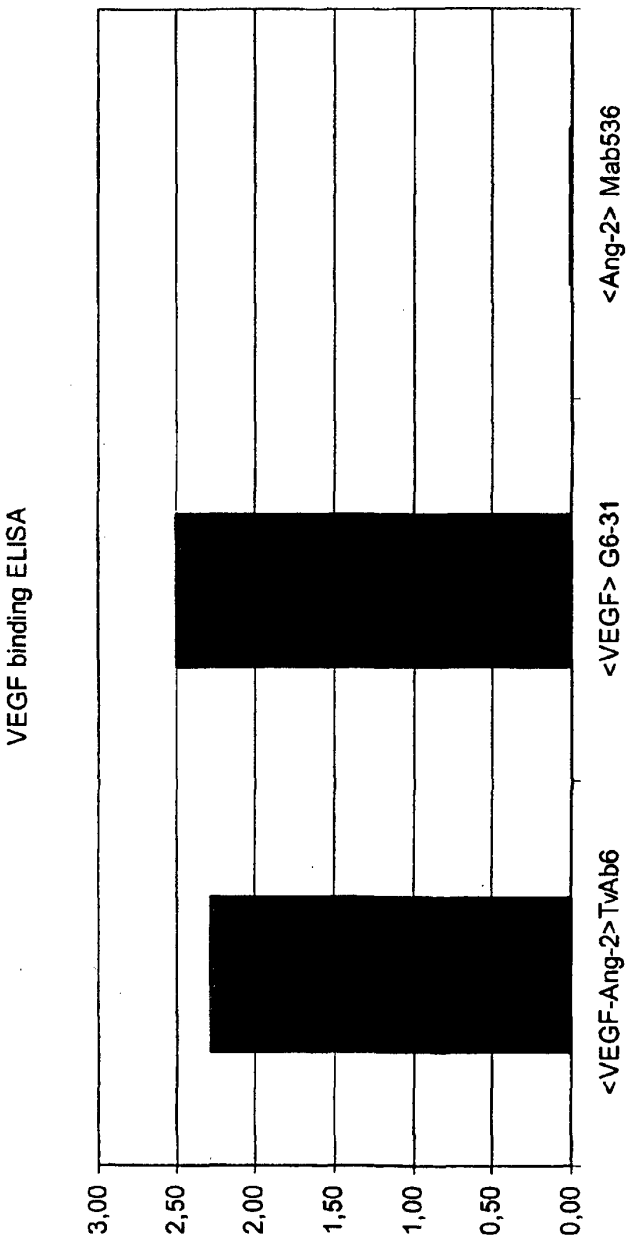


Figure 5–part two

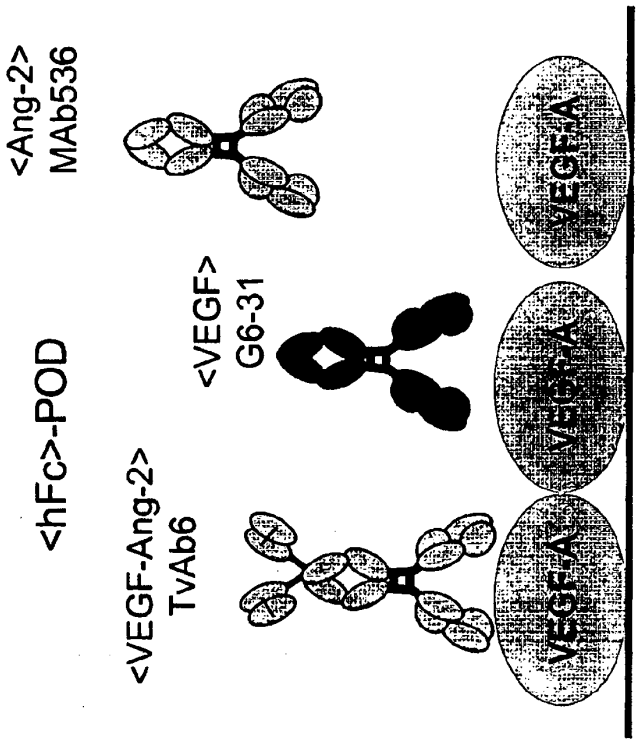
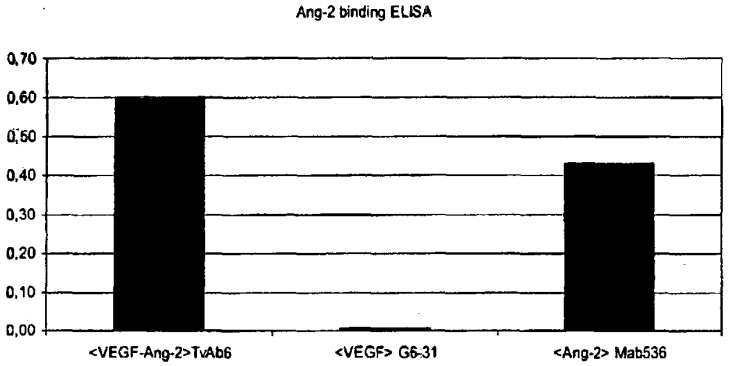


Figure 6A


Ang-2 binding ELISA
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
<VEGF-Ang-2>TvAb6
<VEGF> G6-31
<Ang-2> Mab536

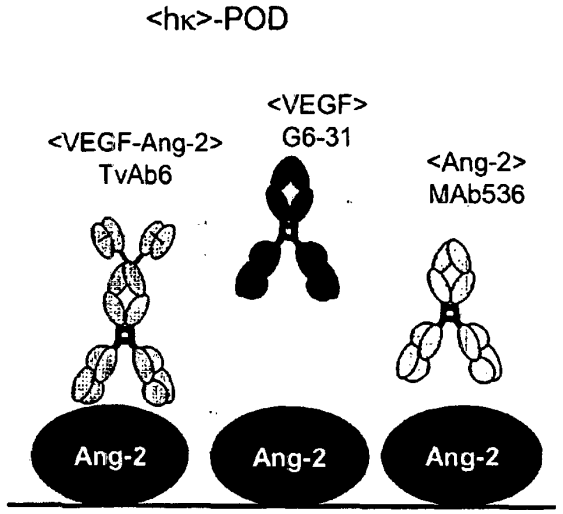

<hκ>-POD
<VEGF-Ang-2>
TvAb6
<VEGF>
G6-31
<Ang-2>
MAb536
Ang-2
Ang-2
Ang-2

Figure 6B

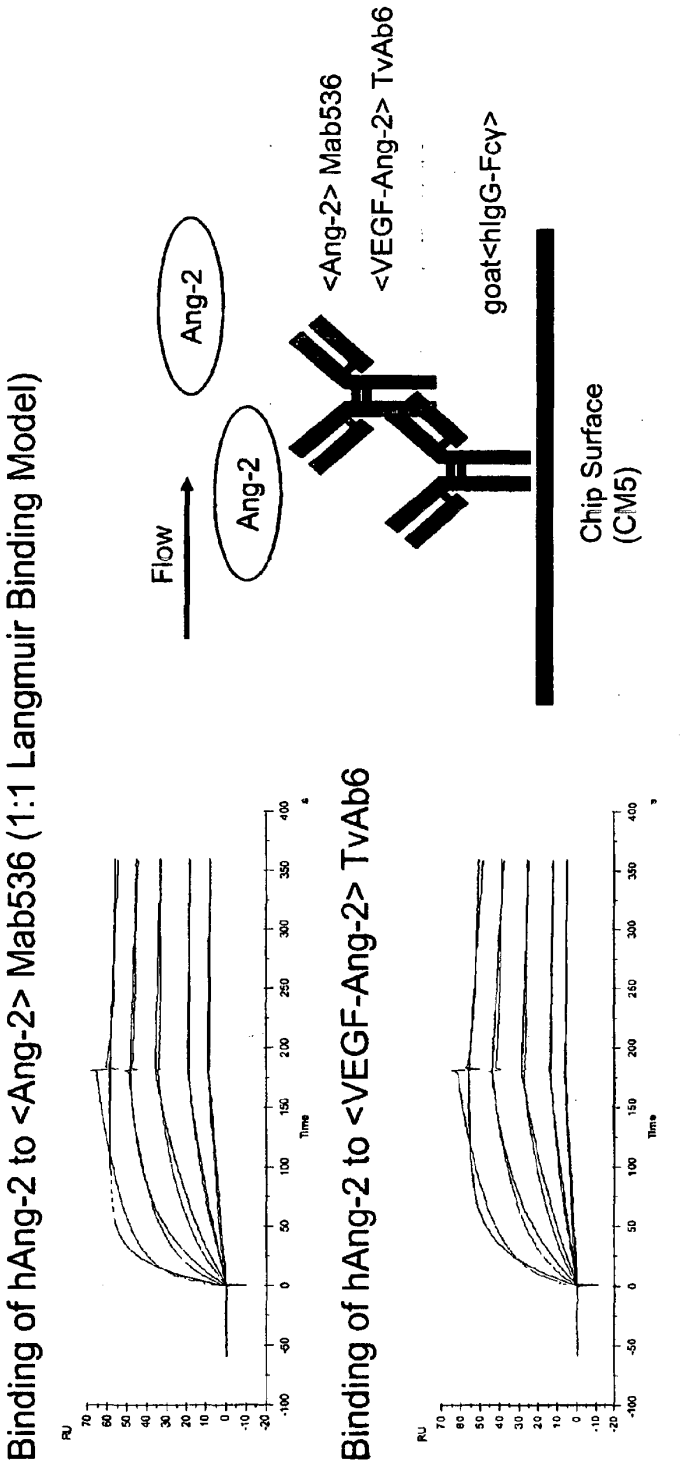

Binding of hAng-2 to <Ang-2> Mab536 (1:1 Langmuir Binding Model)
Flow
Ang-2
Ang-2
<Ang-2> Mab536
<VEGF-Ang-2> TvAb6
goat<hIgG-Fcγ>
Chip Surface
(CM5)
Binding of hAng-2 to <VEGF-Ang-2> TvAb6
RU
Time

Figure 7–part one

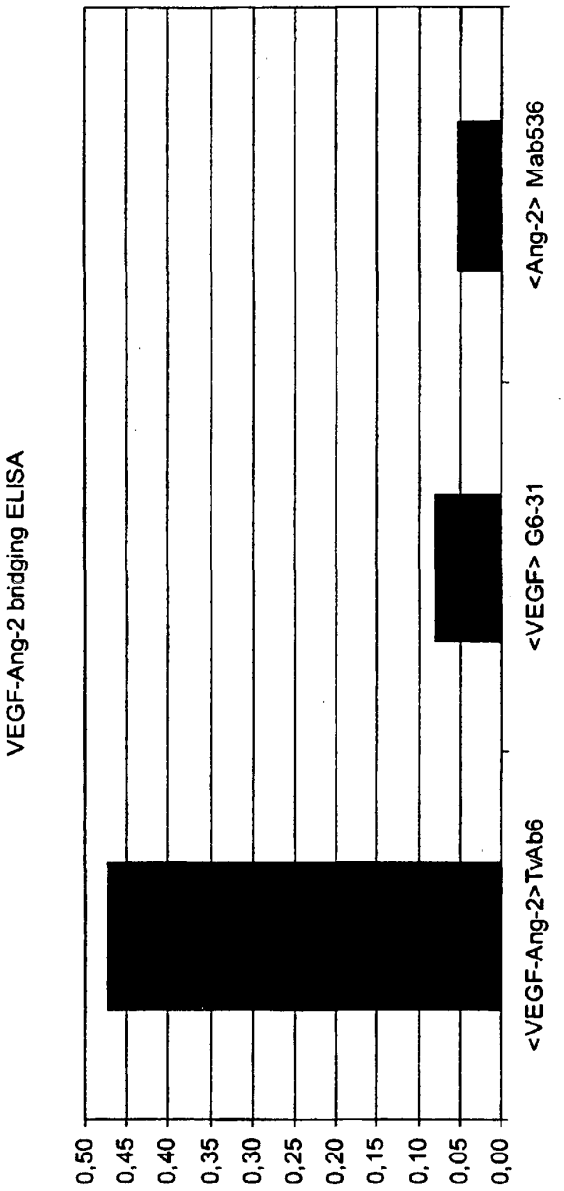


Figure 7–part two

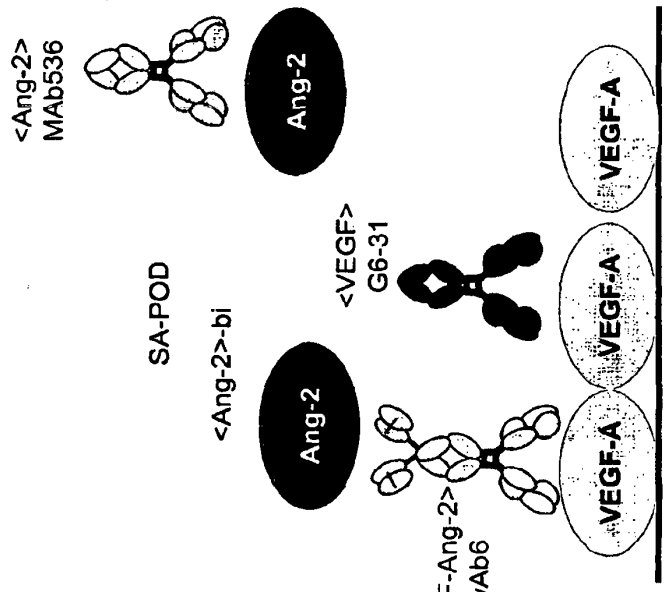


Figure 8a

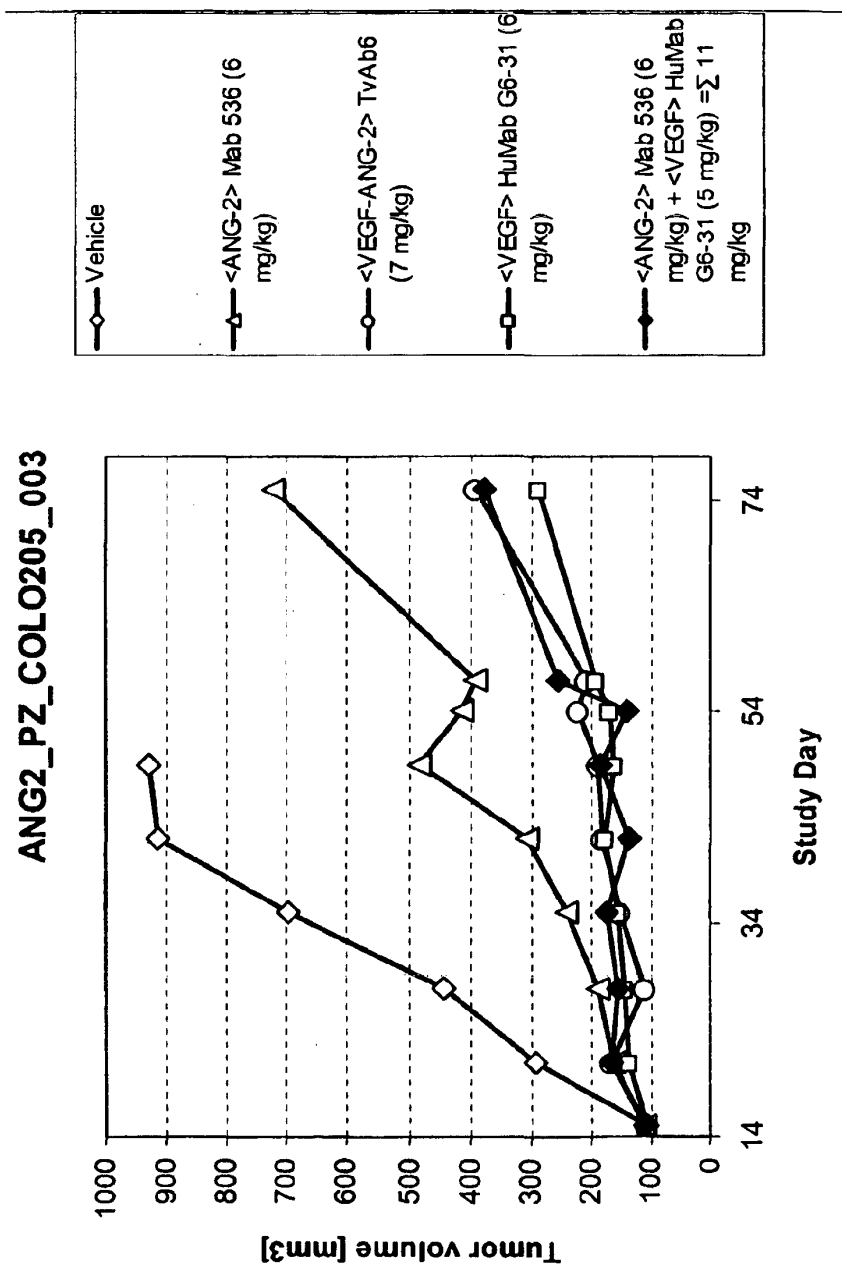

ANG2_PZ_COLO205_003
Tumor volume [mm3]
1000
900
800
700
600
500
400
300
200
100
0
14
34
54
74
Study Day
Vehicle
<ANG-2> Mab 536 (6 mg/kg)
<VEGF-ANG-2> TvAb6 (7 mg/kg)
<VEGF> HuMab G6-31 (6 mg/kg)
<ANG-2> Mab 536 (6 mg/kg) + <VEGF> HuMab G6-31 (5 mg/kg) =Σ 11 mg/kg

Figure 8b

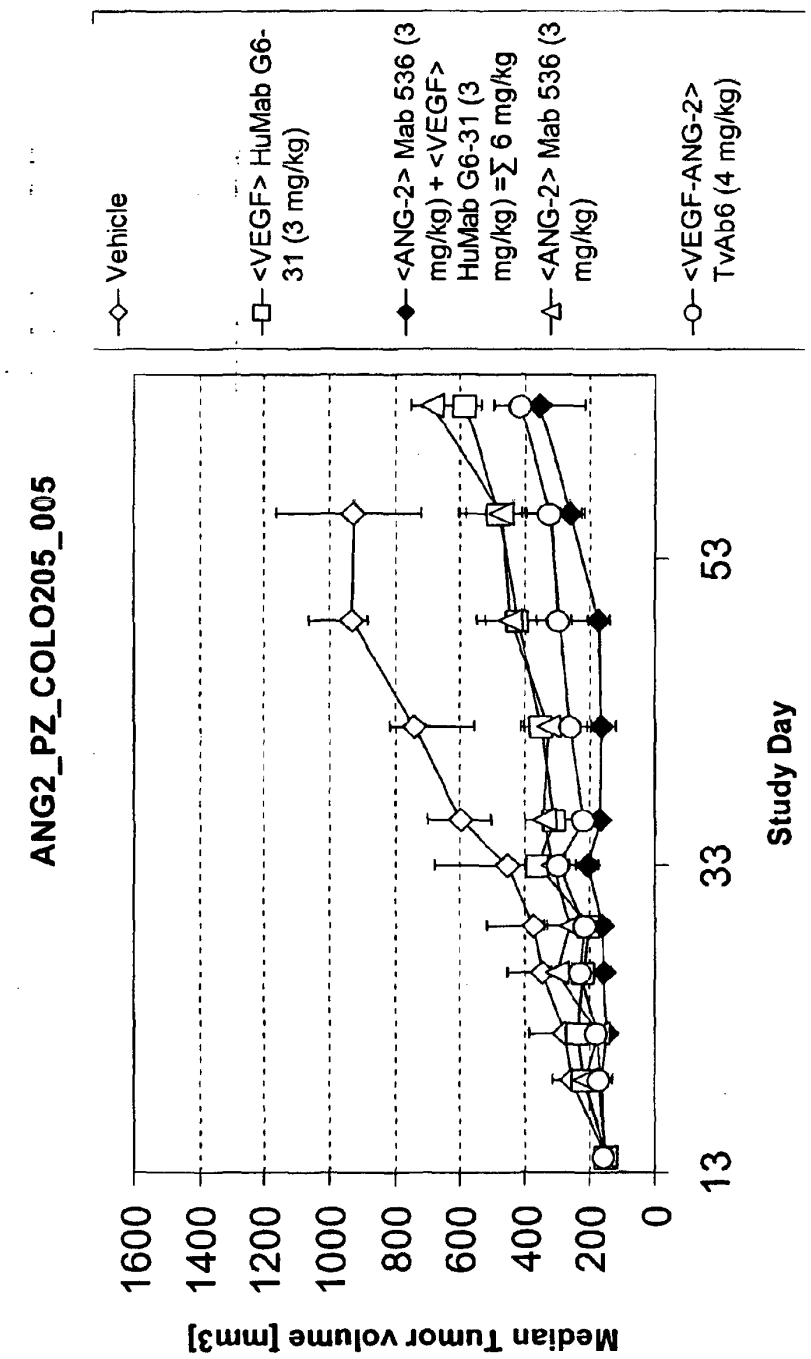

ANG2_PZ_COLO205_005
Median Tumor volume [mm3]
1600
1400
1200
1000
800
600
400
200
0
13
33
53
Study Day
Vehicle
<VEGF> HuMab G6-31 (3 mg/kg)
<ANG-2> Mab 536 (3 mg/kg) + <VEGF> HuMab G6-31 (3 mg/kg) =Σ 6 mg/kg
<ANG-2> Mab 536 (3 mg/kg)
<VEGF-ANG-2> TvAb6 (4 mg/kg)

Figure 9

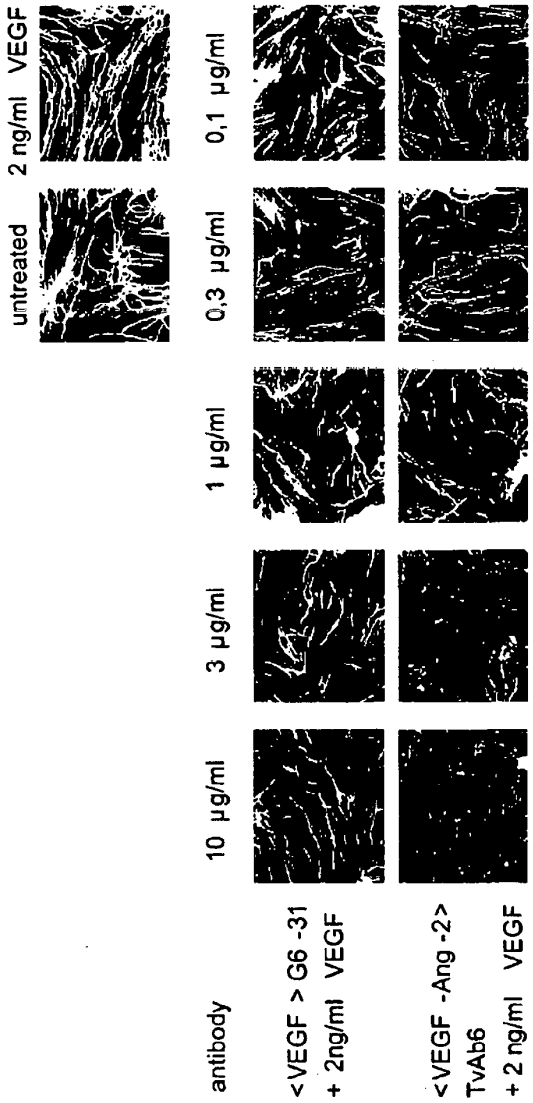

untreated
2 ng/ml VEGF
antibody
10 µg/ml
3 µg/ml
1 µg/ml
0,3 µg/ml
0,1 µg/ml
<VEGF > G6 -31
+ 2ng/ml VEGF
<VEGF -Ang -2>
TvAb6
+ 2 ng/ml VEGF

Figure 10A

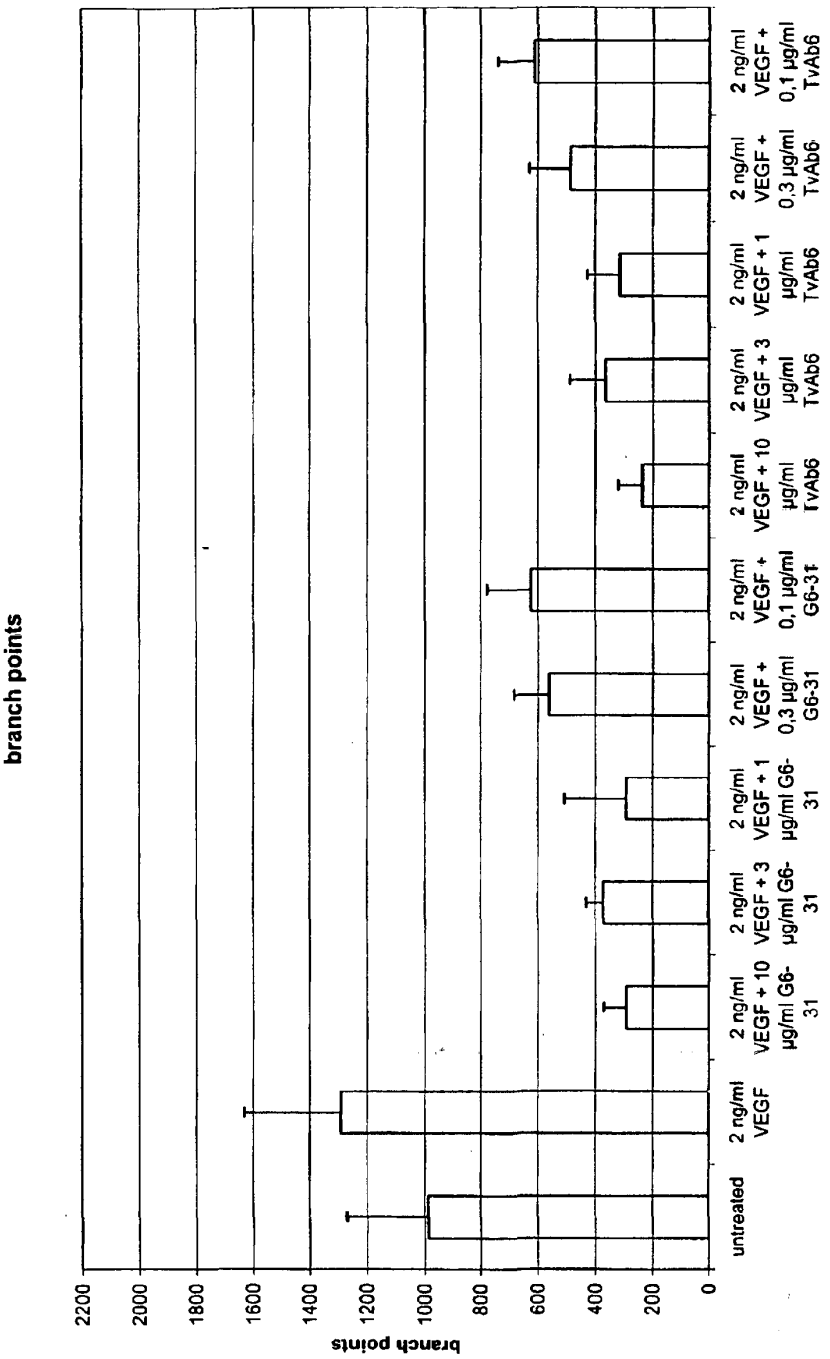

branch points
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
untreated
2 ng/ml VEGF
2 ng/ml VEGF + 10 µg/ml G6-31
2 ng/ml VEGF + 3 µg/ml G6-31
2 ng/ml VEGF + 1 µg/ml G6-31
2 ng/ml VEGF + 0,3 µg/ml G6-31
2 ng/ml VEGF + 0,1 µg/ml G6-31
2 ng/ml VEGF + 10 µg/ml TvAb6
2 ng/ml VEGF + 3 µg/ml TvAb6
2 ng/ml VEGF + 1 µg/ml TvAb6
2 ng/ml VEGF + 0,3 µg/ml TvAb6
2 ng/ml VEGF + 0,1 µg/ml TvAb6

Figure 10B

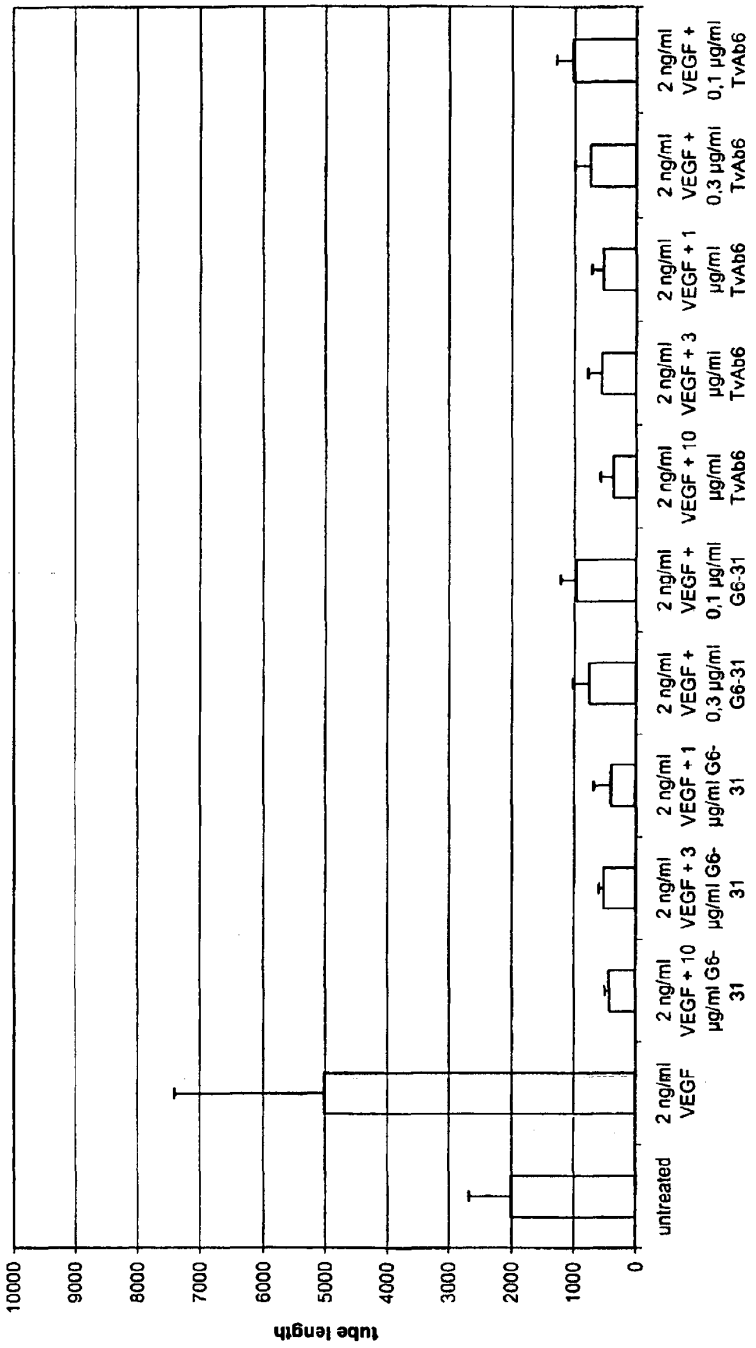

tube length per set
tube length
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
untreated
2 ng/ml VEGF
2 ng/ml VEGF + 10 µg/ml G6-31
2 ng/ml VEGF + 3 µg/ml G6-31
2 ng/ml VEGF + 1 µg/ml G6-31
2 ng/ml VEGF + 0,3 µg/ml G6-31
2 ng/ml VEGF + 0,1 µg/ml G6-31
2 ng/ml VEGF + 10 µg/ml TvAb6
2 ng/ml VEGF + 3 µg/ml TvAb6
2 ng/ml VEGF + 1 µg/ml TvAb6
2 ng/ml VEGF + 0,3 µg/ml TvAb6
2 ng/ml VEGF + 0,1 µg/ml TvAb6

Figure 11

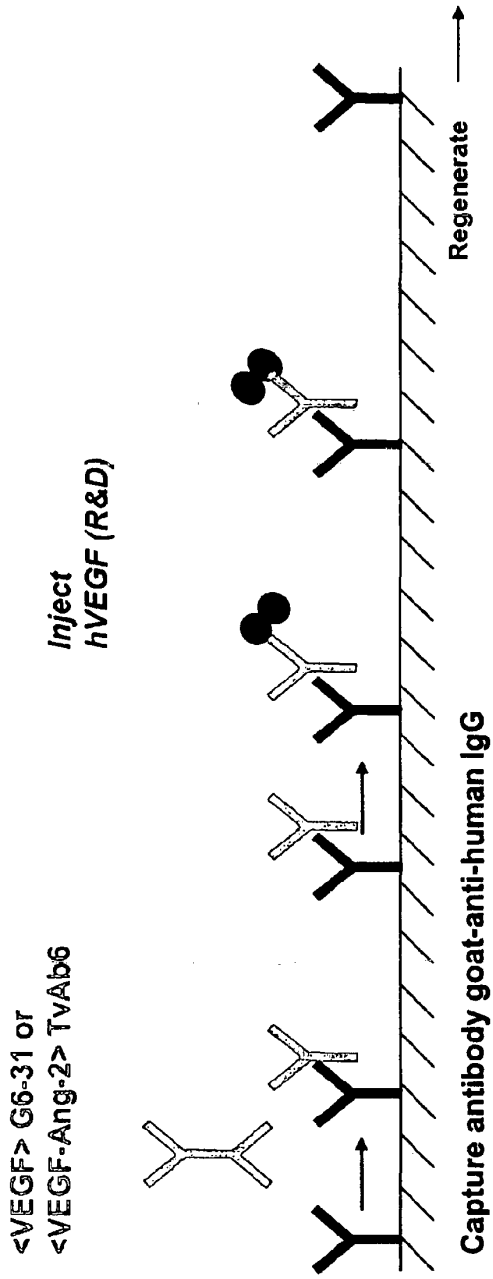

<VEGF> G6-31 or
<VEGF-Ang-2> TvAb6
Inject
hVEGF (R&D)
Capture antibody goat-anti-human IgG
Regenerate

Figure 12

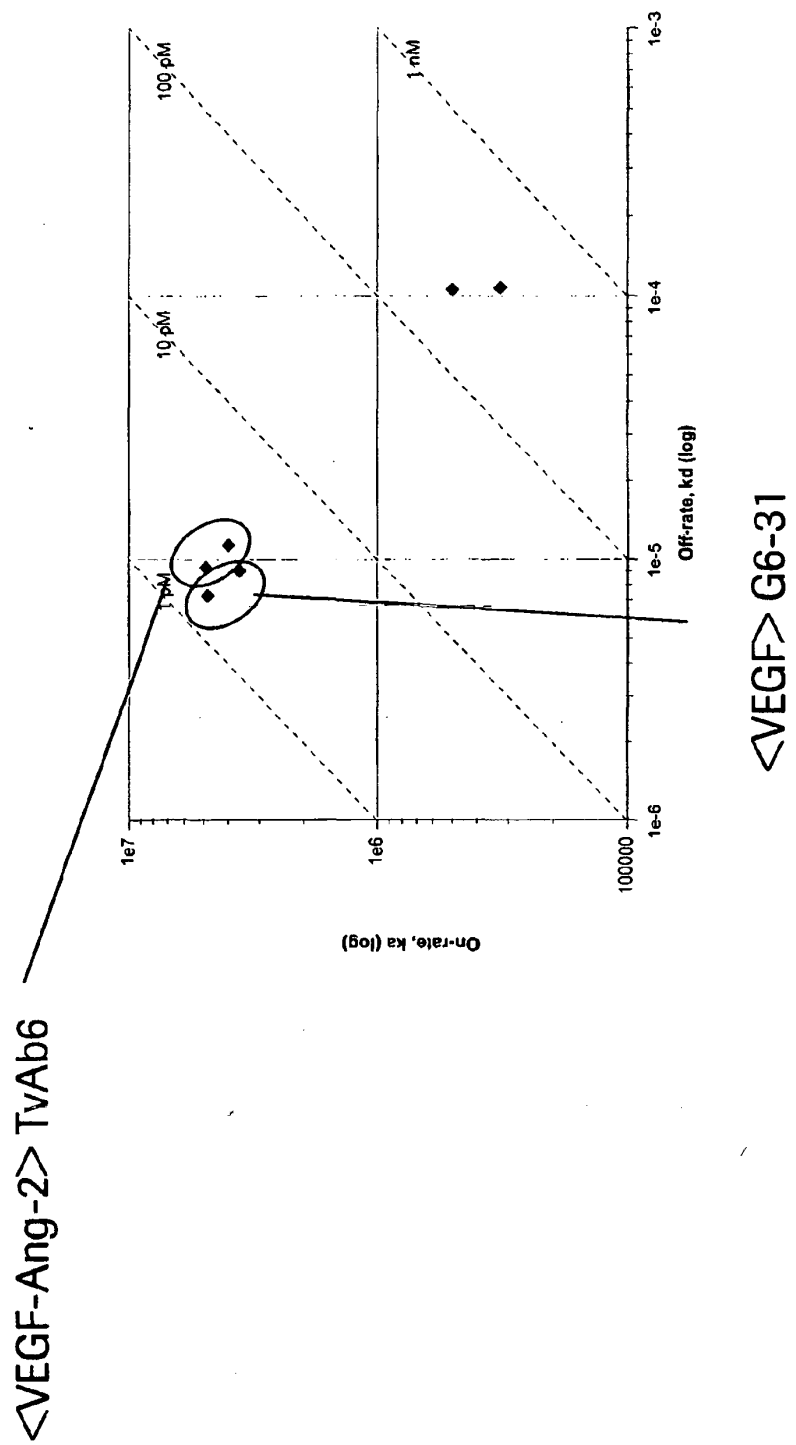

100 pM
1 nM
10 pM
1 pM
1e7
1e6
100000
1e-6
1e-5
1e-4
1e-3
On-rate, ka (log)
Off-rate, kd (log)
<VEGF-Ang-2> TvAb6
<VEGF> G6-31

Figure 13

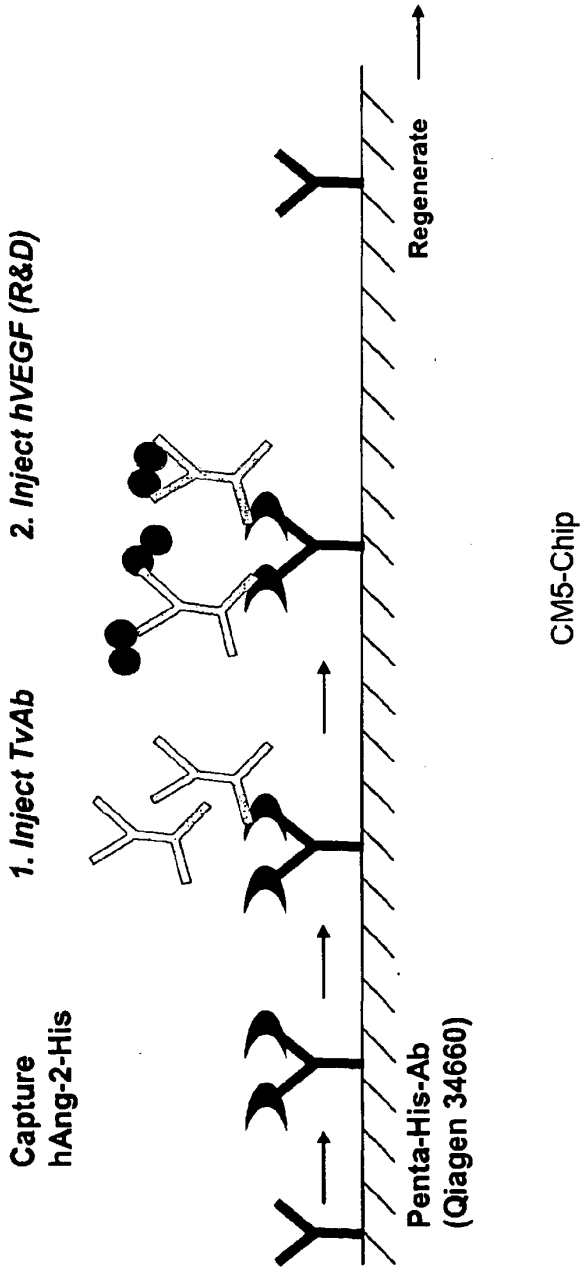

Capture
hAng-2-His
1. Inject TvAb
2. Inject hVEGF (R&D)
Penta-His-Ab
(Qiagen 34660)
Regenerate
CM5-Chip

Figure 14

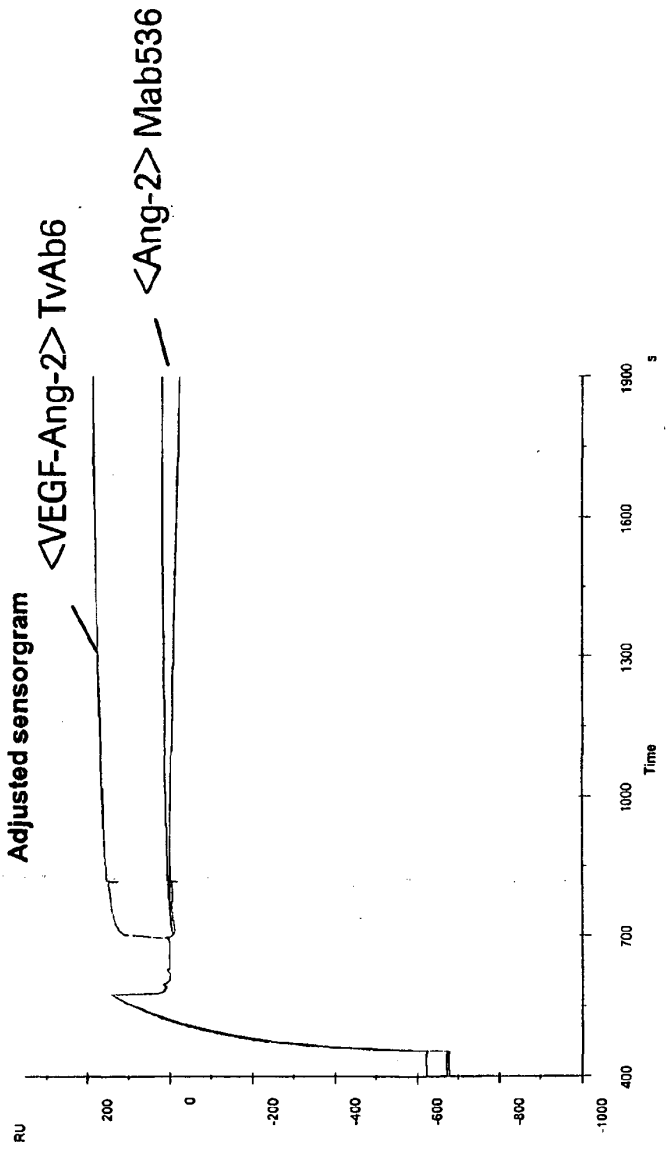

Adjusted sensorgram
<VEGF-Ang-2> TvAb6
<Ang-2> Mab536
RU
200
0
-200
-400
-600
-800
-1000
400
700
1000
1300
1600
1900
Time
s

Fig 15A

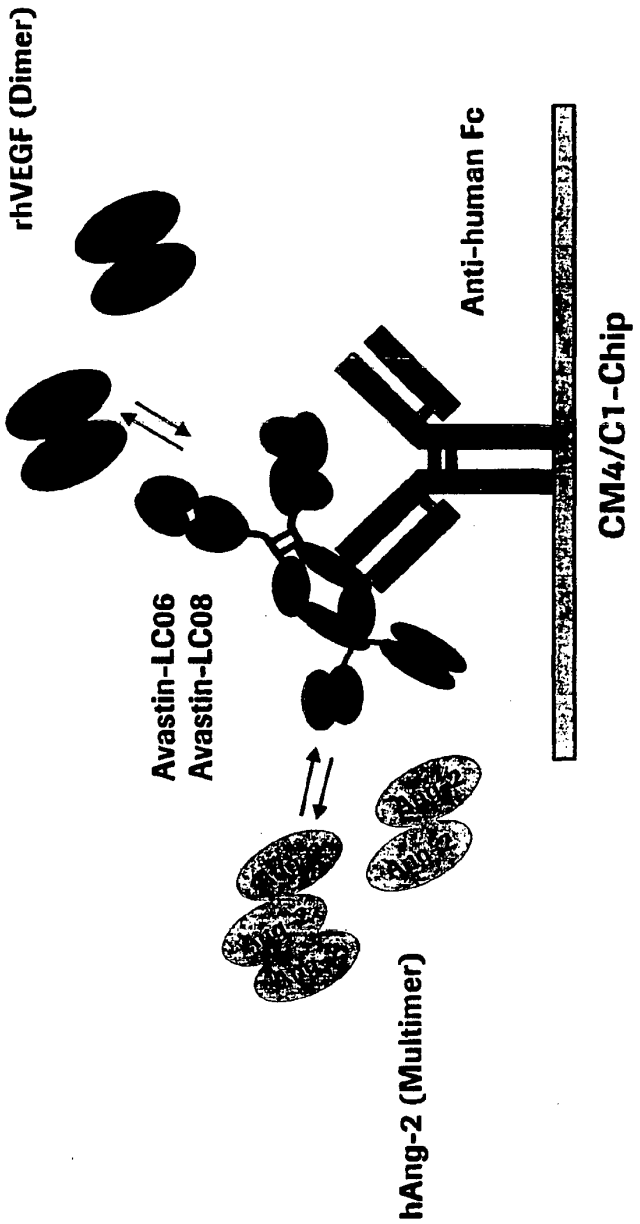

rhVEGF (Dimer)
Anti-human Fc
CM4/C1-Chip
Avastin-LC06
Avastin-LC08
hAng-2 (Multimer)

Fig 15B

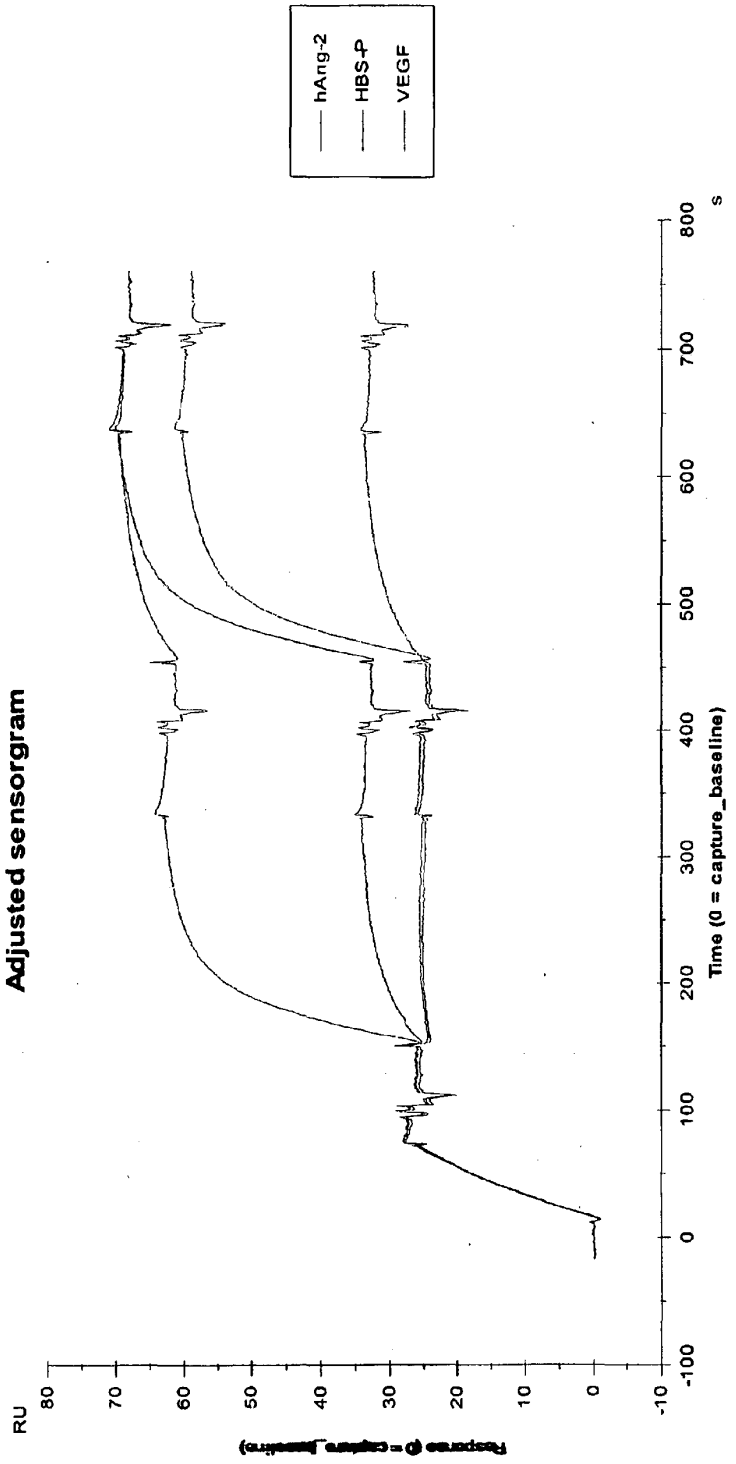

hAng-2
HBS-P
VEGF
Adjusted sensorgram
RU
80
70
60
50
40
30
20
10
0
-10
-100
0
100
200
300
400
500
600
700
800
s
Time (0 = capture_baseline)

Fig 16A

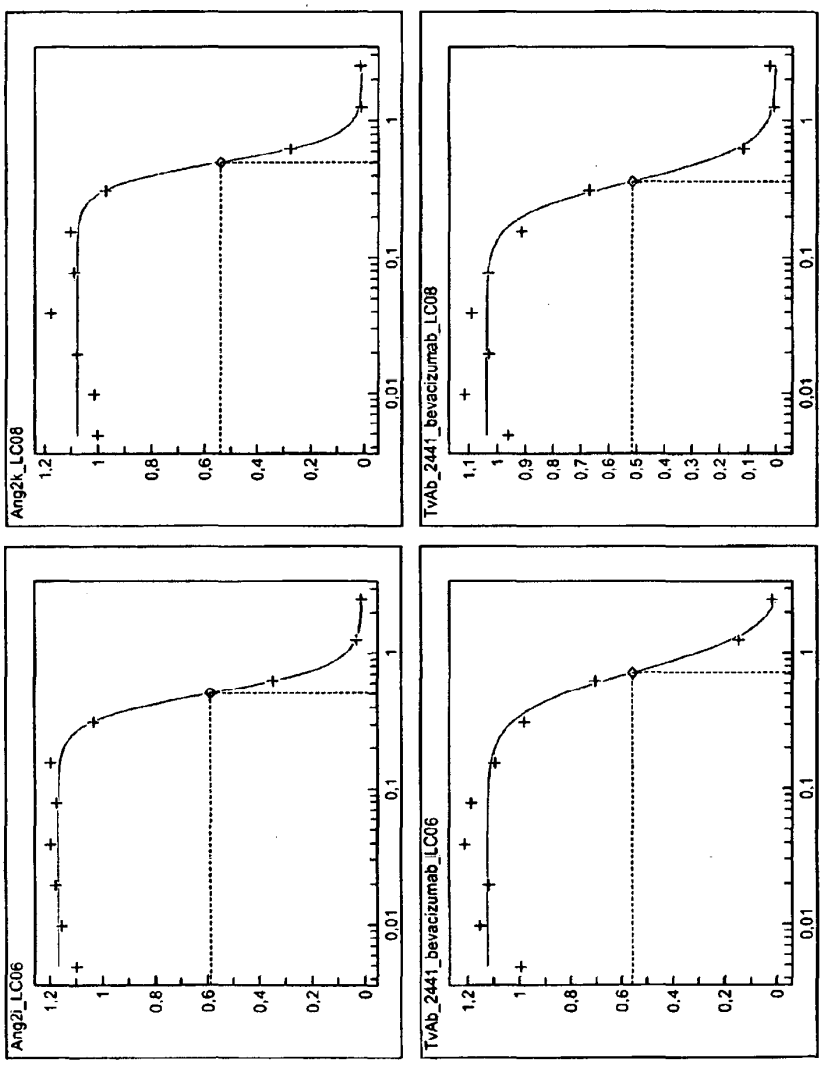

Ang2k_LC08
TvAb_2441_bevacizumab_LC08
Ang2i_LC06
TvAb_2441_bevacizumab_LC06

Fig 16B

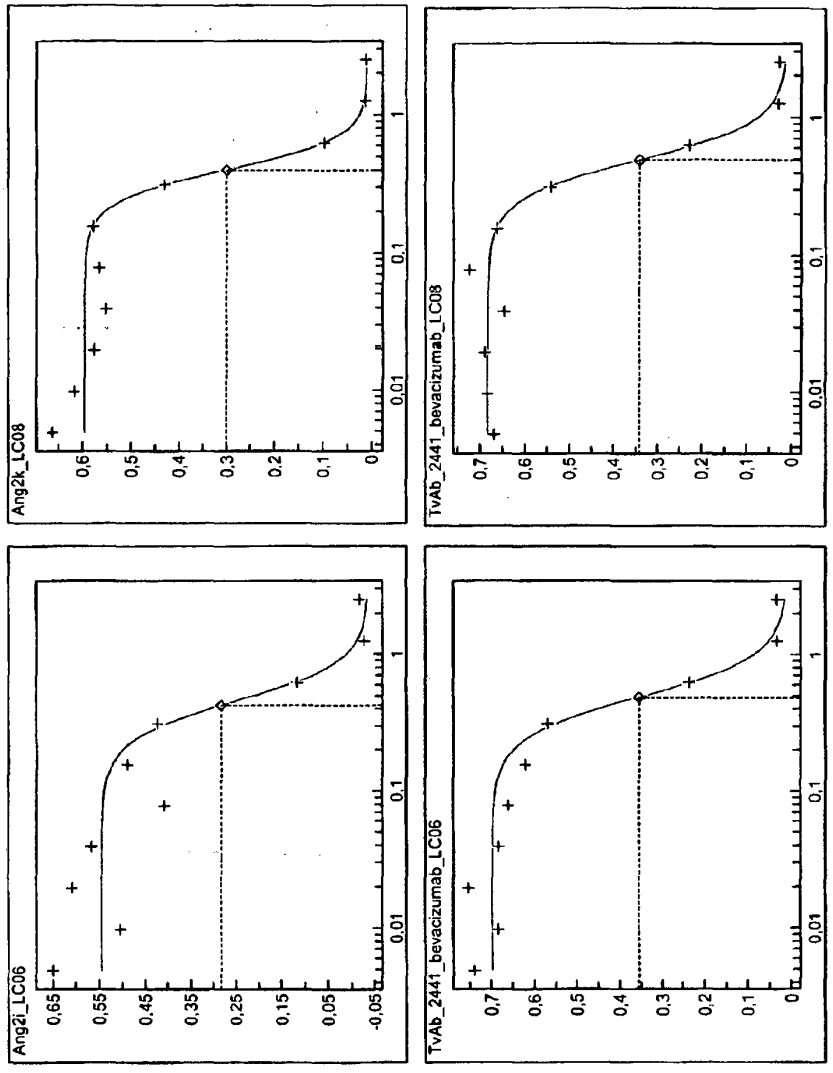
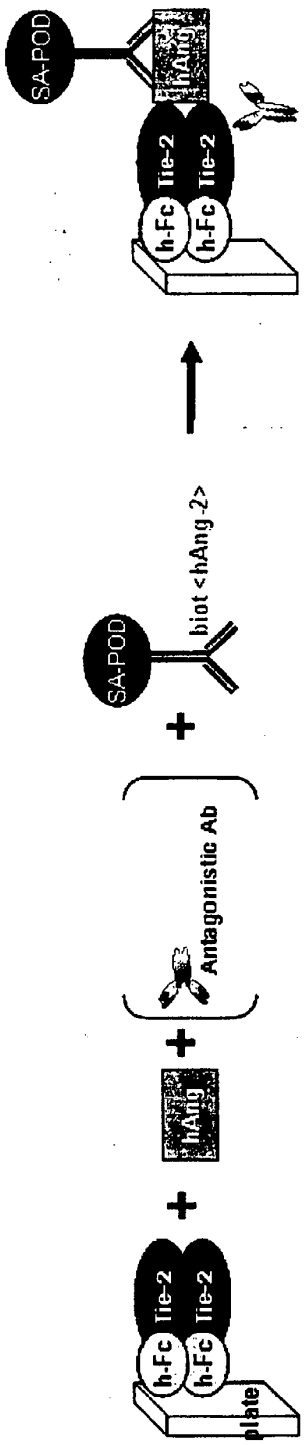

Ang2i_LC06
Ang2k_LC08
TvAb_2441_bevacizumab_LC06
TvAb_2441_bevacizumab_LC08

Fig 17


SA-POD
hAng
Tie-2
Tie-2
h-Fc
h-Fc
biot <hAng-2>
Antagonistic Ab
plate

202

Fig 18

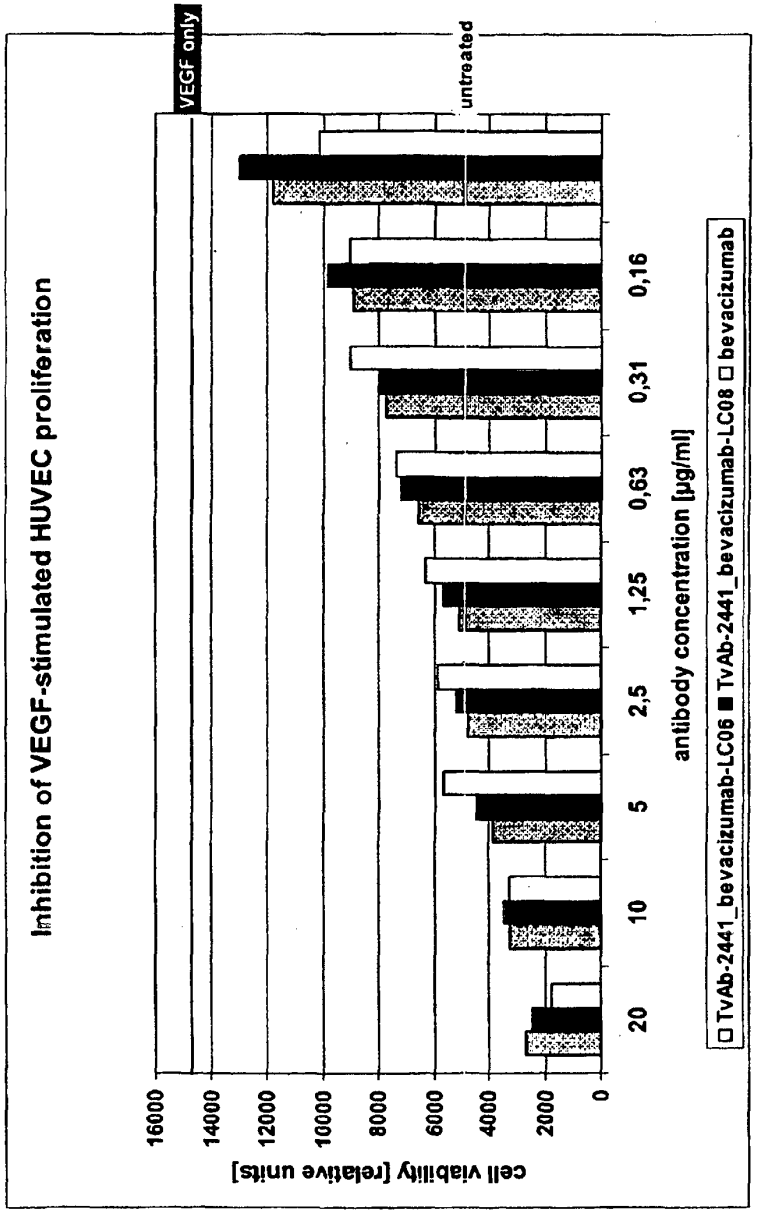
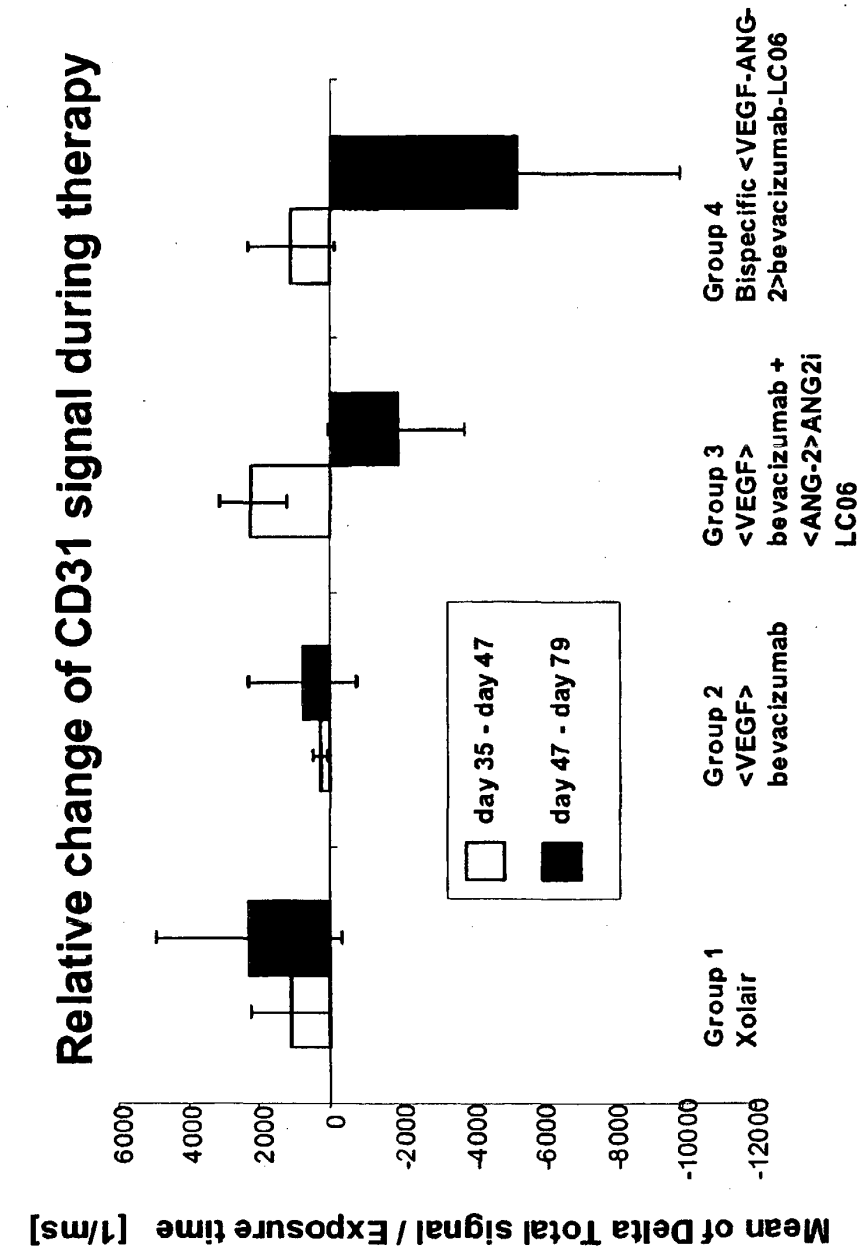

Inhibition of VEGF-stimulated HUVEC proliferation
VEGF only
untreated
cell viability [relative units]
16000
14000
12000
10000
8000
6000
4000
2000
0
20
10
5
2,5
1,25
0,63
0,31
0,16
antibody concentration [µg/ml]
TvAb-2441_bevacizumab-LC06
TvAb-2441_bevacizumab-LC08
bevacizumab

Fig 19


Relative change of CD31 signal during therapy
Mean of Delta Total signal / Exposure time [1/ms]
6000
4000
2000
0
-2000
-4000
-6000
-8000
-10000
-12000
day 35 - day 47
day 47 - day 79
Group 1
Xolair
Group 2
<VEGF>
bevacizumab
Group 3
<VEGF>
bevacizumab +
<ANG-2>ANG2i
LC06
Group 4
Bispecific <VEGF-ANG-2>bevacizumab-LC06

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

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EFS ID:	25076092
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International Application Number:	
Confirmation Number:	1006
Title of Invention:	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES
First Named Inventor/Applicant Name:	Robert L. VITTI
Customer Number:	96387
Filer:	Karl Bozicevic
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Electronically Filed 3/2/2016

INFORMATION DISCLOSURE STATEMENT Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket	REGN-020
	First Named Inventor	Vitti, Robert L.
	Application Number	14/943,490
	Confirmation No.	1006
	Filing Date	November 17, 2015
	Group Art Unit	
	Examiner Name	
	Title: "*Methods and Formulations for Treating Vascular Eye Diseases*"	

Sir:

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

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

Statements

☒ **No statement**

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

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

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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-020.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: March 2, 2016 By: /Karl Bozicevic, Reg. No. 28,807/
Karl Bozicevic
Reg. No. 28,807

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, California 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

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

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OC000000080139886

Date Mailed: 01/28/2016

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

Inventor(s)

Robert L. VITTI, Old Tappan, NJ;
Kristine A. ERICKSON, Roxbury, CT;
Karen W. CHU, White Plains, NY;
Stanley J. WIEGAND, Hopewell Junction, NY;
Jingtai CAO, White Plains, NY;
Ivan B. LOBOV, New York, NY;
Saurabh WADHWA, Elmsford, NY;
Kenneth S. GRAHAM, Pleasant Valley, NY;
Daniel DIX, LaGrangeville, 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 appln claims benefit of 62/084,003 11/25/2014
and claims benefit of 62/147,232 04/14/2015

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

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: No

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:

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/943,490**

Projected Publication Date: 05/26/2016

Non-Publication Request: No

Early Publication Request: No

Title

METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES

Preliminary Class

514

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

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

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

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

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

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

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

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

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

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

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

NOT GRANTED

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

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor

community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/943,490	11/17/2015	Robert L. VITTI	REGN-020

CONFIRMATION NO. 1006

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

37 CFR 1.48(f)
ACKNOWLEDGEMENT LETTER

OC000000080139888

Date Mailed: 01/28/2016

NOTICE OF ACCEPTANCE OF REQUEST UNDER 37 CFR 1.48(f)

This is in response to the applicant's request under 37 CFR 1.48(f) submitted on 01/21/2016.

The request under 37 CFR 1.48(f) to correct the inventorship, to correct or update the name of an inventor, or to correct the order of names of joint inventors is accepted.

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**.

/lqchau/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/943,490	11/17/2015	Robert L. VITTI	REGN-020

CONFIRMATION NO. 1006

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

INFORMAL NOTICE

OC000000080139970

Date Mailed: 01/28/2016

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Robert L. VITTI
  Kristine A. ERICKSON
  Karen W. CHU
  Stanley J. WIEGAND
  Jingtai CAO
  Ivan B. LOBOV
  Saurabh WADHWA
  Kenneth S. GRAHAM
  Daniel DIX

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**.

/lqchau/

Filed Electronically January 21, 2016

REQUEST FOR CORRECTED FILING RECEIPT Address to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket	REGN-020
	Applicant	Vitti, Robert L.
	Serial Number	14/943,490
	Filing Date	November 17, 2015
	Group Art Unit	
	Examiner Name	
	Title: *"Methods and Formulations for Treating Vascular Eye Diseases"*	

Sir:

A filing receipt for the above-identified patent application has been issued by the U.S. Patent and Trademark Office (copy attached) and has been found to contain the following error(s):

(1) Please correct the last name of inventor "WEIGAND" to read --WIEGAND-- as indicated on the attached Supplemental Application Data Sheet.

If for any reason a fee is found to be necessary, the Commissioner is authorized to charge such fee to Deposit Account No. 50-0815.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 21 January 2016

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

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, California 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/943,490	11/17/2015	1629	2540	REGN-020	30	1

CONFIRMATION NO. 1006

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

FILING RECEIPT

OC000000078935797

KWZ/
KYB

Date Mailed: 12/01/2015

RECEIVED
By mse at 8:31 am, Dec 01, 2015

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

Inventor(s)

Robert L. VITTI, Old Tappan, NJ;
Kristine A. ERICKSON, Roxbury, CT;
Karen W. CHU, White Plains, NY;
Stanley J. ~~WEIGAND~~, Hopewell Junction, NY; **WIEGAND**
Jingtai CAO, White Plains, NY;
Ivan B. LOBOV, New York, NY;
Saurabh WADHWA, Elmsford, NY;
Kenneth S. GRAHAM, Pleasant Valley, NY;
Daniel DIX, LaGrangeville, 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 appln claims benefit of 62/084,003 11/25/2014
and claims benefit of 62/147,232 04/14/2015

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Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: No

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: 11/30/2015

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/943,490**

Projected Publication Date: 05/26/2016

Non-Publication Request: No

Early Publication Request: No

Title

METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES

Preliminary Class

514

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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NOT GRANTED

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community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

PTO/AIA/14 (07-14)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

SUPPLEMENTAL

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-020
	Application Number	14/943,490
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

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
	Robert	L.	VITTI	

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

City	Old Tappan	State/Province	NJ	Country of Residence	US

Mailing Address of Inventor:

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

City	Tarrytown	State/Province	NY
Postal Code	10591	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Kristine	A.	ERICKSON	

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

City	Roxbury	State/Province	CT	Country of Residence	US

Mailing Address of Inventor:

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

City	Tarrytown	State/Province	NY
Postal Code	10591	Country	US

Inventor 3 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Karen	W.	CHU	

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

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

SUPPLEMENTAL

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	~~14/943,490~~
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

City	White Plains	State/Province	NY	Country of Residence	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	State/Province	NY
Postal Code	10591	Country	US

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Stanley	J.	~~WEIGAND~~ WIEGAND	

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

City	Hopewell Junction	State/Province	NY	Country of Residence	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	State/Province	NY
Postal Code	10591	Country	US

Inventor 5 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Jingtai		CAO	

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

City	White Plains	State/Province	NY	Country of Residence	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	State/Province	NY
Postal Code	10591	Country	US

Inventor 6 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Ivan	B.	LOBOV	

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

City	New York	State/Province	NY	Country of Residence	US

PTO/AIA/14 (07-14)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

SUPPLEMENTAL

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	14/943,490
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	**State/Province**	NY
Postal Code	10591	**Country**	US

Inventor 7 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Saurabh		WADHWA	

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

City	Elmsford	**State/Province**	NY	**Country of Residence**	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	**State/Province**	NY
Postal Code	10591	**Country**	US

Inventor 8 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Kenneth	S.	GRAHAM	

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

City	Pleasant Valley	**State/Province**	NY	**Country of Residence**	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	**State/Province**	NY
Postal Code	10591	**Country**	US

Inventor 9 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Daniel		DIX	

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

City	LaGrangeville	**State/Province**	NY	**Country of Residence**	US

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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-020
	Application Number	14/943,490
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

Mailing Address of Inventor:			
Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	**State/Province**	NY
Postal Code	10591	**Country**	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	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES		
Attorney Docket Number	REGN-020	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	4	**Suggested Figure for Publication (if any)**	1

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.

PTO/AIA/14 (07-14)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

SUPPLEMENTAL

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-020
	Application Number	14/943,490
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

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 this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Claims benefit of provisional	62084003	2014-11-25
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Claims benefit of provisional	62147232	2015-04-14

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

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[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.

PTO/AIA/14 (07-14)
Approved for use through 04/30/2017. OMB 0651-0032
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SUPPLEMENTAL

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	14/943,490
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

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

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

Authorization to Permit Access:

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

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

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

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

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.

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Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

SUPPLEMENTAL

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	14/943,490
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

Applicant 1

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

Clear

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

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

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	REGENERON PHARMACEUTICALS, INC.

Mailing Address Information For Applicant:

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

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

Assignee Information including Non-Applicant Assignee Information:

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

Assignee 1

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

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

PTO/AIA/14 (07-14)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

SUPPLEMENTAL

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-020
	Application Number	14/943,490
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

Organization Name	REGENERON PHARMACEUTICALS, INC.

Mailing Address Information For Assignee including Non-Applicant Assignee:

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

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

Signature:

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

Signature	/Karl Bozicevic, Reg. No. 28,807/			Date (YYYY-MM-DD)	2016-01-21 ~~2015-11-17~~
First Name	Karl	Last Name	Bozicevic	Registration Number	28807

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

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.**

SUPPLEMENTAL

Privacy Act Statement

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

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

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

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 C o o p eration Treaty.

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

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

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

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt	
EFS ID:	24690995
Application Number:	14943490
International Application Number:	
Confirmation Number:	1006
Title of Invention:	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES
First Named Inventor/Applicant Name:	Robert L. VITTI
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-020
Receipt Date:	21-JAN-2016
Filing Date:	17-NOV-2015
Time Stamp:	18:11:59
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Corrected Filing Receipt	REGN-020_01-21-2016_request_correct_OFR.pdf	23132 3ae63c32ceff2d94c77376f20c7788d2ae48fed7	no	1
Warnings:					
Information:					

2	Request for Corrected Filing Receipt	REGN-020_OFR_mark-up.pdf	1232451 119de358bf40443ba2e8a23430fde001514bf176	no	4
Warnings:					
Information:					
3	Application Data Sheet	REGN-020_supp_ads.pdf	1205430 beb50d0dd37ecaae82daaa30dce3d9837ff4fb50	no	9
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
			Total Files Size (in bytes):	2461013	

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

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/943,490	11/17/2015	1629	2540	REGN-020	30	1

CONFIRMATION NO. 1006

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

FILING RECEIPT

OC000000078935797

Date Mailed: 12/01/2015

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

Inventor(s)

Robert L. VITTI, Old Tappan, NJ;
Kristine A. ERICKSON, Roxbury, CT;
Karen W. CHU, White Plains, NY;
Stanley J. WEIGAND, Hopewell Junction, NY;
Jingtai CAO, White Plains, NY;
Ivan B. LOBOV, New York, NY;
Saurabh WADHWA, Elmsford, NY;
Kenneth S. GRAHAM, Pleasant Valley, NY;
Daniel DIX, LaGrangeville, 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 appln claims benefit of 62/084,003 11/25/2014
and claims benefit of 62/147,232 04/14/2015

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

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: No

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: 11/30/2015

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/943,490**

Projected Publication Date: 05/26/2016

Non-Publication Request: No

Early Publication Request: No

Title

METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES

Preliminary Class

514

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

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

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

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

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

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

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

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

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

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

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

NOT GRANTED

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

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor

community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 14/943,490

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	30 minus 20 =	* 10			OR	x 80 =	800
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*				x 420 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	2400

APPLICATION AS AMENDED - PART II

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

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

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

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

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/943,490	11/17/2015	Robert L. VITTI	REGN-020

CONFIRMATION NO. 1006

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

INFORMAL NOTICE

OC000000078955798

Date Mailed: 12/01/2015

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Robert L. VITTI
  Kristine A. ERICKSON
  Karen W. CHU
  Stanley J. WEIGAND
  Jingtai CAO
  Ivan B. LOBOV
  Saurabh WADHWA
  Kenneth S. GRAHAM
  Daniel DIX

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**.

/afessehaye/

UNITED STATES PATENT AND TRADEMARK OFFICE

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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/943,490	11/17/2015	Robert L. VITTI	REGN-020

CONFIRMATION NO. 1006

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

IMPROPER LETTER

OC000000078955801

Date Mailed: 12/01/2015

NOTICE OF ACCEPTANCE OF AUTHORIZATION TO PERMIT ACCESS TO APPLICATION VIA PRIORITY DOCUMENT EXCHANGE

This is in response to the applicant's authorization to permit access to the application-as-filed by participating offices under 37 CFR 1.14(h)(1) submitted on 11/17/2015.

The authorization to permit access to the application under 37 CFR 1.14(h)(1) is accepted.

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**.

/afessehaye/

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Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).
Reviewer: Douglas, Keisha (ASRC)
Timestamp: [year=2015; month=11; day=25; hr=14; min=17; sec=19; ms=475;
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Validated By CRFValidator v 1.0.4

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Robert L. Vitti
Kristine A. Erickson
Karen W. Chu
Stanley J. Weigand
Jingtai Cao
Ivan B. Lobov
Saurabh Wadhwa
Kenneth S. Graham
Daniel Dix

<120> METHODS AND FORMULATIONS FOR TREATING
VASCULAR EYE DISEASES

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Electronically Filed

AMENDMENT UNDER 37 C.F.R. §1.115 Address to: Mail Stop Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket No.	REGN-020
	Confirmation No.	1006
	First Named Inventor	Vitti, Robert L.
	Application Number	14/943,490
	Filing Date	17 November 2015
	Group Art Unit	
	Examiner Name	
	Title: "*Methods and Formulations for Treating Vascular Eye Diseases*"	

Sir:

Prior to examination of the application, Applicants submit the following amendments . In view of the amendments to the claims and the remarks put forth below, reconsideration and allowance are respectfully requested.

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

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS:

1. -92. (Canceled)

93. (Original) A method for treating a vascular eye disease, the method comprising sequentially administering one or more doses of a pharmaceutical composition comprising therapeutically active amount of an anti-Ang-2 inhibitor and a VEGF antagonist to a subject in need thereof.

94. (Original) The method of claim 93, wherein the pharmaceutical composition comprises about 10 mg/mL to about 120 mg/mL of the anti-Ang-2 inhibitor.

95. (Original) The method of claim 93, wherein the pharmaceutical composition comprises about 40 mg/mL of the VEGF antagonist.

96. (Original) The method of claim 93, wherein the pharmaceutical composition comprises about 10 mg/mL to about 120 mg/mL of the anti-Ang-2 inhibitor and about 40 mg/mL of the VEGF antagonist.

97. (Original) The method of claim 93, wherein the pharmaceutical composition comprises 10 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

98. (Original) The method of claim 93, wherein the pharmaceutical composition comprises 20 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

99. (Original) The method of claim 93, wherein the pharmaceutical composition comprises 60 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

100. (Original) The method of claim 93, wherein the pharmaceutical composition comprises 120 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

101. (Original) The method of claim 93 comprising administering an initial dose of the pharmaceutical composition to the subject; followed by administering one or more secondary doses of

the pharmaceutical composition to the subject, wherein each secondary dose is administered 1 to 4 weeks after the immediately preceding dose.

102. (Original) The method of claim 101, wherein at least 2 secondary doses are administered to the subject and each secondary dose is administered 4 weeks after the immediately preceding dose.

103. (Original) The method of claim 102 further comprising administering one or more tertiary doses of the pharmaceutical composition to the subject wherein each tertiary dose is administered 5 to 12 weeks after the immediately preceding dose.

104. (Original) The method of claim 103, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

105. (Original) The method of claim 93, wherein each dose of the pharmaceutical composition comprises about 0.5mg to about 10mg of the anti-Ang-2 inhibitor and about 2mg of the VEGF antagonist.

106. (Original) The method of claim 105, wherein each dose of the pharmaceutical composition comprises 10 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

107. (Original) The method of claim 105, wherein each dose of the pharmaceutical composition comprises 20 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

108. (Original) The method of claim 105, wherein each dose of the pharmaceutical composition comprises 60 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

109. (Original) The method of claim 105, wherein each dose of the pharmaceutical composition comprises 120 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

110. (Original) The method of claim 93, wherein each dose of the pharmaceutical composition is intravitreally administered to the subject.

111. (Original) The method of claim 93, wherein the vascular eye disease is selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, central retinal vein occlusion, branched retinal vein occlusion, and polypoidal choroidal vasculopathy.

112. (Original) The method of claim 111, wherein the vascular eye disease is age-related macular degeneration.

113. (Original) The method of claim 111, wherein the vascular eye disease is diabetic macular edema.

114. (Original) The method of claim 93, wherein the Ang-2 inhibitor is an anti-Ang-2 antibody or antigen binding fragment thereof.

115. (Original) The method of claim 114, wherein the antibody or antigen binding fragment comprises the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO: 1 and the CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO: 2.

116. (Original) The method of claim 114, wherein the antibody or antigen binding fragment comprises a heavy chain CDR1 (HCDR1) having the amino acid sequence of SEQ ID NO: 3, a HCDR2 having the amino acid sequence of SEQ ID NO: 4, a HCDR3 having the amino acid sequence of SEQ ID NO: 5, a light chain CDR1 (LCDR1) having the amino acid sequence of SEQ ID NO: 6, a LCDR2 having the amino acid sequence of SEQ ID NO: 7, and a LCDR3 having the amino acid sequence of SEQ ID NO: 8.

117. (Original) The method of claim 116, wherein the antibody or antigen binding fragment thereof comprises a HCVR having the amino acid sequence of SEQ ID NO: 1 and a LCVR having the amino acid sequence of SEQ ID NO: 2.

118. (Original) The method of claim 93, wherein the VEGF antagonist comprises a VEGF receptor-based chimeric molecule (VEGF Trap).

119. (Original) The method of claim 118, wherein the VEGF Trap comprises one or more immunoglobulin (Ig)-like domains of VEGFR1, one or more Ig-like domains of VEGFR2, and a multimerizing domain.

120. (Original) The method of claim 119, wherein the VEGF Trap comprises Ig-like domain 2 of VEGFR1, Ig-like domain 3 of VEGFR2, and a multimerizing domain.

121. (Original) The method of claim 120, wherein the VEGF Trap is aflibercept.

122. (Original) The method of claim 93, wherein the VEGF antagonist consists of a dimer of two polypeptides consisting of amino acids 27-457 of SEQ ID NO: 11.

REMARKS

FORMAL MATTERS:

Claims 93-122 are pending after entry of the amendments set forth herein.

Claims 1-92 are canceled without prejudice.

No claims are amended.

No new matter is added.

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-020.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 18 November 2015

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

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, California 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

Electronic Patent Application Fee Transmittal	
Application Number:	14943490
Filing Date:	
Title of Invention:	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES
First Named Inventor/Applicant Name:	Robert L. VITTI
Filer:	Karl Bozicevic/Kimberly Zuehlke
Attorney Docket Number:	REGN-020
Filed as Large Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
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Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Pages:				
Claims:				
Claims in Excess of 20	1202	10	80	800
Miscellaneous-Filing:				
Late Filing Fee for Oath or Declaration	1051	1	140	140

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
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Electronic Acknowledgement Receipt	
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Application Number:	14943490
International Application Number:	
Confirmation Number:	1006
Title of Invention:	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES
First Named Inventor/Applicant Name:	Robert L. VITTI
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-020
Receipt Date:	18-NOV-2015
Filing Date:	
Time Stamp:	12:15:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$2540
RAM confirmation Number	9407
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-020_preliminary_amendment.pdf	41540 a1a663ba72c17a6cd8bfef9d12de32a18202512e	yes	6

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Preliminary Amendment	1	1
Claims	2	5
Applicant Arguments/Remarks Made in an Amendment	6	6

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	38871 36b84dd08636343d9e11807f8edadb16d9ea5d8e	no	2

Warnings:

Information:

Total Files Size (in bytes):	80411

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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.

DocCode – SEQ.TXT

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Application Number: 14943490 Document Date: 11/17/2015

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Form Revision Date: August 26, 2013

Electronically Filed November 17, 2015

CERTIFICATION REGARDING SEQUENCE LISTING Address to: Mail Stop Patent Application Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket	REGN-020
	First Named Inventor	Vitti, Robert L.
	Application Number	To Be Assigned
	Filing Date	17 November 2015
	Confirmation Number	To Be Assigned
	Group Art Unit	
	Examiner Name	
	Title: "METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES"	

Sir:

Applicants submit herewith the attached Sequence Listing in .txt format. As set out in MPEP §502.05, IX(A), the Office has advised that if the sequence listing text file submitted via EFS-Web complies with the requirements of 37 CFR 1.824(a)(2)-(6) and (b) (i.e., is a compliant sequence listing ASCII text file), the text file will serve as both the paper copy required by 37 CFR 1.821(c) and the computer readable form (CRF) required by 37 CFR 1.821(e). Further, per MPEP §502.05, IX(A), neither (1) a second copy of the sequence listing in a PDF file; nor (2) a statement under 37 CFR 1.821(f) (indicating that the paper copy and CRF copy of the sequence listing are identical) should be submitted.

Please insert the attached "Sequence Listing" as separately numbered pages 1-7 after the abstract.

The present application has been amended to insert the Sequence Listing.

No new matter is added.

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

Applicants respectfully submit that the present patent application is now in compliance with 37 CFR §§ 1.821 - 1.825. 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-020.

Respectfully submitted,

BOZICEVIC, FIELD & FRANCIS LLP

Dated: 17 November 2015

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

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, California 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

Electronic Acknowledgement Receipt	
EFS ID:	24104462
Application Number:	14943490
International Application Number:	
Confirmation Number:	1006
Title of Invention:	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES
First Named Inventor/Applicant Name:	Robert L. VITTI
Customer Number:	96387
Filer:	Karl Bozicevic
Filer Authorized By:	
Attorney Docket Number:	REGN-020
Receipt Date:	17-NOV-2015
Filing Date:	
Time Stamp:	15:07:28
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	Application Data Sheet	REGN-020_10112US01_ads.pdf	1819751 d3987b7c670f9b3faf46bb491abe9769fbd6a0f1	no	9
Warnings:					
Information:					

2		REGN-020_10112US01_spec_as_fld.pdf	628539 f73bba09a751c2e5c5ee422eae0d7af9a5ed326d	yes	107

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Specification	1	94
	Claims	95	106
	Drawings-only black and white line drawings	107	107

Warnings:					
Information:					
3	Drawings-only black and white line drawings	REGN-020_10112_drawings.pdf	58501 cfca383c82b86a9beb7f1c4c8ebc37bf94063c40	no	4
Warnings:					
Information:					
4	Miscellaneous Incoming Letter	REGN-020_10112US01_seq_list_trans.pdf	51291 912ab0b5261e6b8977bade1f0160831e48a6dd88	no	2
Warnings:					
Information:					
5	Sequence Listing (Text File)	REGN-020_10112US01_seqlisting_Text.txt	13027	no	0
Warnings:					
Information:					
		Total Files Size (in bytes):	2571109		

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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 Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

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
	Robert	L.	VITTI	

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

City	Old Tappan	State/Province	NJ	Country of Residence i	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	State/Province	NY
Postal Code	10591	Country i	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Kristine	A.	ERICKSON	

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

City	Roxbury	State/Province	CT	Country of Residence i	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	State/Province	NY
Postal Code	10591	Country i	US

Inventor 3 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Karen	W.	CHU	

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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

City	White Plains	State/Province	NY	Country of Residence i	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	State/Province	NY
Postal Code	10591	Country i	US

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Stanley	J.	WEIGAND	

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

City	Hopewell Junction	State/Province	NY	Country of Residence i	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	State/Province	NY
Postal Code	10591	Country i	US

Inventor 5 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Jingtai		CAO	

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

City	White Plains	State/Province	NY	Country of Residence i	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	State/Province	NY
Postal Code	10591	Country i	US

Inventor 6 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Ivan	B.	LOBOV	

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

City	New York	State/Province	NY	Country of Residence i	US

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	**State/Province**	NY
Postal Code	10591	**Country** i	US

Inventor 7 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Saurabh		WADHWA	

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

City	Elmsford	**State/Province**	NY	**Country of Residence** i	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	**State/Province**	NY
Postal Code	10591	**Country** i	US

Inventor 8 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Kenneth	S.	GRAHAM	

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

City	Pleasant Valley	**State/Province**	NY	**Country of Residence** i	US

Mailing Address of Inventor:

Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	**State/Province**	NY
Postal Code	10591	**Country** i	US

Inventor 9 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Daniel		DIX	

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

City	LaGrangeville	**State/Province**	NY	**Country of Residence** i	US

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

Mailing Address of Inventor:			
Address 1	c/o Regeneron Pharmaceuticals, Inc.		
Address 2	777 Old Saw Mill River Road		
City	Tarrytown	**State/Province**	NY
Postal Code	10591	**Country** i	US

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

Correspondence Information:

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

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

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

Application Information:

Title of the Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES		
Attorney Docket Number	REGN-020	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	4	**Suggested Figure for Publication (if any)**	1

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 i

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.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

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 this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Claims benefit of provisional	62084003	2014-11-25
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Claims benefit of provisional	62147232	2015-04-14

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

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) [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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

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

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

Authorization to Permit Access:

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

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

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

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

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.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

Applicant 1 Remove

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

Clear

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

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

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	REGENERON PHARMACEUTICALS, INC.		
Mailing Address Information For Applicant:			
Address 1	777 Old Saw Mill River Road		
Address 2			
City	Tarrytown	**State/Province**	NY
Country [i]	US	Postal Code	10591
Phone Number		Fax Number	
Email Address			

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

Assignee Information including Non-Applicant Assignee Information:

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

Assignee 1

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

Remove

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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	REGN-020
	Application Number	
Title of Invention	METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES	

Organization Name	REGENERON PHARMACEUTICALS, INC.

Mailing Address Information For Assignee including Non-Applicant Assignee:

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

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

Signature:

Remove

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

Signature	/Karl Bozicevic, Reg. No. 28,807/			Date (YYYY-MM-DD)	2015-11-17
First Name	Karl	Last Name	Bozicevic	Registration Number	28807

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

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.**

Privacy Act Statement

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

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

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

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 C o o p eration Treaty.

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

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

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

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

METHODS AND FORMULATIONS FOR TREATING VASCULAR EYE DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit under 35 U.S.C. § 119(e) of US provisional application Nos. 62/084,003, filed on November 25, 2014; and 62/147,232, filed on April 14, 2015, the disclosures of each herein incorporated by reference in their entireties.

FIELD OF THE INVENTION

[0001] The present invention relates to methods of treating or ameliorating at least one symptom or indication of a vascular eye disease comprising administering a pharmaceutical formulation comprising an angiopoietin-2 (Ang-2) inhibitor and a vascular endothelial growth factor (VEGF) antagonist to a subject in need thereof.

BACKGROUND

[0002] Vascular eye diseases are the leading cause of vision loss in today's aging population. These diseases are characterized by abnormal 'leaky' blood vessels growing into the retina. Two of the largest contributors to this patient population are diabetic retinopathy and exudative age-related macular degeneration.

[0003] Diabetic retinopathy (DR) is a major cause of visual impairment in the United States (Klein et al 1984, Ophthalmology 91:1464-1474; Moss et al 1998, Ophthalmology 105:998-1003). Diabetic retinopathy results from microvascular decompensation beginning with basement membrane thickening (Ruggiero et al 1997, Diabetes Metab. 23:30–42), and eventually leading to vascular occlusion and neovascularization (Porta et al 2002, Diabetologia. 45:1617–1634). It is estimated that about 28% of patients 40 years and older with diabetes have DR, and 4.4% have vision threatening DR (Zhang et al 2010, JAMA. 304: 649-656). Diabetic macular edema (DME) is a manifestation of DR and is the most frequent cause of blindness in young and mid-aged adults (Klein et al 1984, Ophthalmology 91:1464-1474; Moss et al 1998, Ophthalmology 105:998-1003).

[0004] Age-related macular degeneration (AMD) is the leading cause of severe visual loss in people aged 50 years or older in the developed world. In recent years, major advances have been made in the treatment of AMD, with the introduction of anti-angiogenic agents, offering hope of significant visual recovery for patients with neovascular AMD (Keane et al 2012, Surv Ophthalmol. 57: 389-414).

[0005] Anti-vascular endothelial growth factor (VEGF) therapy (*e.g.*, aflibercept) is standard of care treatment for neovascular AMD and DME. The efficacy and safety of aflibercept in these patient populations is well-characterized (Dixon et al 2009; Expert Opin. Investig. Drugs 18: 1573-

80). However, in AMD, although ~95% of patients maintained their vision, only approximately 30% of patients achieved an improvement of 15 or more letters in best corrected visual acuity (BCVA) at 1 year. In DME, there is also the possibility of improving treatment outcomes, as seen with aflibercept and with ranibizumab, less than 50% of patients with vision loss due to DME achieve a 15 or more letter improvement over 1 and 2 years. Also, in the studies with ranibizumab, clinical evidence of proliferative retinopathy developed in up to 7.2% of patients who had received 3 years of monthly treatment of ranibizumab, with up to 3.2% of patients requiring panretinal photocoagulation, a potentially visually disabling treatment modality (Brown et al 2013 Ophthalmology 10: 2013-22).

[0006] Intravitreal (IVT) deliveries of anti-VEGF therapies such as ranibizumab and aflibercept have demonstrated efficacy and safety for chorioretinal diseases. However, there are many additional factors that contribute to vascular permeability, neovascularization, and other vascular dysfunction. One of the most studied factors that contribute to vascular permeability is angiopoietin-2 (Ang-2). Ang-2 is expressed by vascular endothelial cells during normal vascular development and also in the course of physiological or pathological angiogenesis in the adult (Maisonpierre et al 1997, Science 277: 55-60; Holash et al 1999, Science 284: 1994-98). Binding of Ang-2 to its receptor Tie-2 promotes angiogenesis, both during normal vascular development and in conditions characterized by pathological neovascularization. Genetic deletion of Ang-2 in the mouse markedly inhibits both normal retinal vascular development and pathological neovascularization (Hackett et al 2000, J. Cell Physiol. 184: 275-83; Hackett et al 2002, J. Cell Physiol. 192: 182-7; Gale et al 2002, Dev. Cell 3: 411-423). Targeting angiopoietin-2 (Ang2) will support inhibition of any of these factors, either alone or in combination, and has the potential to improve upon the success of anti-VEGF therapy alone.

BRIEF SUMMARY OF THE INVENTION

[0007] According to one aspect of the present invention, methods are provided for treating, preventing or ameliorating at least one symptom or indication of a vascular eye disease or disorder in a subject. The methods according to this aspect of the invention comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an angiopoietin-2 (Ang-2) inhibitor to a subject in need thereof. In certain embodiments, the Ang-2 inhibitor is administered in combination with a vascular endothelial growth factor (VEGF) antagonist.

[0008] According to another aspect of the present invention, methods are provided for inhibiting retinal angiogenesis in a subject. The methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor in combination with a VEGF antagonist to the subject in need thereof. In certain embodiments, combined administration results

in reduction of the retinal vascular area by at least 65% as compared to the administration of either Ang-2 inhibitor or the VEGF antagonist alone. In some embodiments, the retinal angiogenesis is associated with a vascular eye disease or disorder.

[0009] In another aspect, the present invention provides for methods for inhibiting retinal neovascularization in a subject with an eye disease or disorder associated with angiogenesis, the methods comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor in combination with a VEGF antagonist to the subject in need thereof.

[0010] According to another aspect of the present invention, methods are provided for inhibiting vascular leak in a subject with an eye disease or disorder. The methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor in combination with a VEGF antagonist to a subject in need thereof.

[0011] In a related aspect, the present invention provides methods for suppressing vascular leak in a subject with an eye disease or disorder associated with angiogenesis, wherein the methods comprise administering a single dose of a VEGF antagonist followed by one or more doses of a pharmaceutical composition comprising an Ang-2 inhibitor to the subject in need thereof.

[0012] In several embodiments, the vascular leak is inhibited for at least 3 weeks, more than 3 weeks, more than 4 weeks, more than 8 weeks, or more than 10 weeks as compared to a subject who has been administered the VEGF antagonist alone.

[0013] In another aspect, the present invention provides for methods for inhibiting choroidal neovascularization comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor to a subject in need thereof.

[0014] According to another aspect of the present invention, methods are provided for reducing the dependence and treatment burden of frequent intravitreal injections in a subject with a vascular eye disease or disorder. The methods comprise sequentially administering an initial dose followed by one or more secondary doses of a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor in combination with a VEGF antagonist to the subject in need thereof; wherein the administration of the pharmaceutical composition is reduced to once every 9 weeks as compared to a subject who has been administered the VEGF antagonist alone.

[0015] In certain embodiments, the present invention provides method for treating a vascular eye disease, the methods comprising administering one or more doses of a pharmaceutical composition comprising a therapeutically active amount of an anti-Ang-2 inhibitor and a therapeutically active amount of a VEGF antagonist to a subject in need thereof. In certain embodiments, the methods comprise administering an initial dose of the pharmaceutical composition followed by one or more secondary doses. In certain embodiments, each secondary dose is administered 1 to 4 weeks after

the immediately preceding dose. In certain embodiments, the methods further comprise administration of one or more tertiary doses to the subject. In certain embodiments, each tertiary dose is administered 5 to 12 weeks after the immediately preceding dose. In one embodiment, each secondary dose is administered 4 weeks after the immediately preceding dose. In certain embodiments, each tertiary dose is administered 8 weeks or 12 weeks after the immediately preceding dose. In certain embodiments, the pharmaceutical composition comprises about 10 mg/mL to about 120 mg/mL of the anti-Ang-2 inhibitor. In certain embodiments, the pharmaceutical composition comprises about 40 mg/mL of the VEGF antagonist. In certain embodiments, the pharmaceutical composition comprises about 10 mg/mL to about 120 mg/mL of the anti-Ang-2 inhibitor and about 40 mg/mL of the VEGF antagonist. In certain embodiments, the pharmaceutical composition is intravitreally administered to the subject. In certain embodiments, each dose of the pharmaceutical composition comprises about 0.5mg to about 6mg of the anti-Ang-2 inhibitor and about 2mg of the VEGF antagonist. In one embodiment, each dose of the pharmaceutical composition comprises about 3mg of the anti-Ang-2 inhibitor and about 2mg of the VEGF antagonist. In one embodiment, each dose of the pharmaceutical composition comprises about 6mg of the anti-Ang-2 inhibitor and about 2mg of the VEGF antagonist.

[0016] In certain embodiments, the eye disease or disorder is selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal neovascularization, central retinal vein occlusion, branched retinal vein occlusion, polypoidal choroidal vasculopathy, and choroidal neovascularization.

[0017] In certain embodiments, the Ang-2 inhibitor is administered as a co-formulation with a VEGF antagonist. In certain embodiments, the Ang-2 inhibitor alone or in combination with the VEGF antagonist is intravitreally administered to a subject in need thereof. In alternate embodiments, the Ang-2 inhibitor is administered intravenously or subcutaneously. In certain embodiments, the Ang-2 inhibitor is administered intravenously or subcutaneously in combination with the VEGF antagonist, wherein the VEGF antagonist is administered intravitreally. In certain embodiments, an Ang-2 inhibitor and a VEGF antagonist are co-administered topically or intraocularly (*e.g.*, intravitreally).

[0018] In certain embodiments, the Ang-2 inhibitor is administered at a dose of from 0.05 mg to 10 mg to a subject in need thereof. In certain embodiments, the VEGF antagonist is administered at a dose of from 0.01 mg to 5 mg to a subject in need thereof. In some embodiments, the Ang-2 inhibitor is administered at a dose of about 1 – 50 mg/kg of the subject's body weight.

[0019] In certain embodiments, one or more secondary doses of the pharmaceutical composition comprising the Ang-2 inhibitor are administered to a subject in need thereof. In some embodiments, the one or more doses comprise at least 2 secondary doses of the pharmaceutical composition. In

certain embodiments, each secondary dose is administered 1 to 4 weeks after the immediately preceding dose.

[0020] Exemplary Ang-2 inhibitors that can be used in the context of the methods of the present invention include, *e.g.*, small molecule chemical inhibitors of Ang-2, or biological agents that target Ang-2. According to certain embodiments, the Ang-2 inhibitor is an antibody or antigen binding protein that binds the Ang-2 ligand and blocks Tie2 signaling. In certain embodiments, the anti-Ang-2 antibody or antigen-binding protein comprises the heavy chain complementarity determining regions (HCDRs) of a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 1 and the light chain CDRs of a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 2.

[0021] VEGF antagonists that may be used in combination with an Ang-2 inhibitor in the compositions and methods of the present invention include anti-VEGF antibodies (*e.g.*, ranibizumab), small molecule VEGF inhibitors (e.g., sunetinib), and VEGF-inhibiting fusion proteins ("VEGF Traps"). An example of a VEGF antagonist that may be used in combination with the anti-Ang-2 antibodies in the methods of treatment of the present invention is aflibercept, a VEGF-inhibiting fusion protein (*see* US 7,087,411).

[0022] In another aspect, the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of an Ang-2 inhibitor and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the pharmaceutical composition further comprises a VEGF antagonist.

[0023] In certain embodiments, the present invention provides use of an anti-Ang-2 antibody or antigen-binding fragment thereof of the invention in the manufacture of a medicament to treat or prevent or ameliorate at least a symptom or indication of an eye disease or disorder in a subject, including humans.

[0024] In certain embodiments, the present invention provides use of an Ang-2 inhibitor of the invention in conjunction with a VEGF antagonist in the manufacture of a medicament to treat an eye disease or disorder in a subject, including humans.

[0025] According to another aspect of the present invention, a stable liquid pharmaceutical formulation is provided, comprising: (i) a VEGF antagonist; (ii) an antibody or antigen-binding fragment thereof that specifically binds to Ang-2; (iii) a buffer; (iv) a non-ionic detergent; (v) a tonicity agent; and (vi) a stabilizer. In one embodiment, a stable ophthalmic formulation is provided, comprising: (i) a VEGF antagonist; (ii) an antibody or antigen-binding fragment thereof that specifically binds to Ang-2; (iii) a buffer; (iv) a non-ionic detergent; (v) a tonicity agent; and (vi) a stabilizer.

[0026] In one embodiment, the VEGF antagonist is provided at a concentration of from 5 mg/mL ± 0.75 mg/mL to about 100 mg/mL ± 15 mg/mL and the anti-Ang-2 antibody is provided at a concentration of from 10 ± 1.5 mg/mL to 120 ± 18.0 mg/mL. In some embodiments, the VEGF antagonist is provided at a concentration of from 10 mg/mL ± 1.5 mg/mL to 80 mg/mL ± 12 mg/mL, or from 20 mg/mL ± 3.0 mg/mL to 60 mg/mL ± 9.0 mg/mL. In one embodiment, the VEGF antagonist is provided at a concentration of 40 mg/mL ± 6.0 mg/mL, or about 40 mg/mL. In one embodiment, the antibody is provided at a concentration of 10 mg/ml ± 1.5 mg/mL, or about 10 mg/mL. In another embodiment, the antibody is provided at a concentration of 20 mg/mL ± 3.0 mg/mL, or about 20 mg/mL. In another embodiment, the antibody is provided at a concentration of 60 mg/mL ± 9.0 mg/mL, or about 60 mg/mL. In another embodiment, the antibody is provided at a concentration of 120 mg/mL ± 18.0 mg/mL, or about 120 mg/mL.

[0027] In certain embodiments, the pH of the liquid formulation is from about pH 5.5 to about pH 6.5. In some embodiments, the pH of the liquid formulation is pH 6.2 ± 0.3, pH 6.2 ± 0.25, pH 6.2 ± 0.2, pH 6.2 ± 0.15, pH 6.2 ± 0.1, pH 6.2 ± 0.05, pH 6.2 ± 0.01, or pH 6.2. In one embodiment, the pH of the liquid formulation is pH 6.2 ± 0.3, or about pH 6.2.

[0028] In one embodiment, the buffer is sodium phosphate. In some embodiments, the sodium phosphate is at a concentration of from 5 mM ± 0.75 mM to 50 mM ± 7.5 mM, 5 mM ± 0.75 mM to 40 mM ± 6.0 mM, or 5 mM ± 0.75 mM to 25 mM ± 3.75 mM. In one embodiment, the sodium phosphate is at a concentration of 10 mM ± 1.5 mM or about 10 mM.

[0029] In some embodiments, the non-ionic detergent is a nonionic polymer containing a polyoxyethylene moiety. In some embodiments, the non-ionic detergent is any one or more of polysorbate 20, poloxamer 188 and polyethylene glycol 3350. In one embodiment, the detergent is polysorbate 20. In one embodiment, the detergent is polysorbate 80.

[0030] In certain embodiments, the non-ionic detergent is at a concentration of from 0.005% ± 0.00075% to 1% ± 0.15% "weight to volume" or "w/v", wherein, *e.g.,* 0.1 g/ml = 10% and 0.01 g/ml = 1%. In one embodiment, the non-ionic detergent is polysorbate 20, which is at a concentration of from about 0.01% ± 0.0045% to about 0.05% ± 0.0045% w/v. In one embodiment, the non-ionic detergent is polysorbate 20, which is at a concentration of 0.03% ± 0.0045% w/v or about 0.03% w/v.

[0031] In some embodiments, the tonicity agent is sodium chloride or potassium chloride. In one embodiment, the tonicity agent is sodium chloride. In some embodiments, the tonicity agent is sodium chloride at a concentration of from 10 mM ± 1.5 mM to 75 mM ± 11.25 mM, 20 mM ± 3.0 mM to 60 mM ± 9.0 mM, or 30 mM ± 4.5 mM to 50 mM ± 7.5 mM. In one embodiment, the sodium chloride is at a concentration of 40 mM ± 6.0 mM, or about 40 mM.

[0032] In one embodiment, the stabilizer is a sugar. In one embodiment, the sugar is selected from the group consisting of sucrose, mannitol and trehalose. In one embodiment, the stabilizer is sucrose.

[0033] In some embodiments, the stabilizer is at a concentration of from 1% ± 0.15% w/v to 20% ± 3% w/v. In some embodiments, the stabilizer is sucrose at a concentration of from 1% ± 0.15% w/v to 15% ± 2.25% w/v, or from 1% ± 0.15% w/v to 10% ± 1.5% w/v. In one embodiment, the stabilizer is sucrose at a concentration of 5% ± 0.15% w/v or about 5% w/v. In another embodiment, the stabilizer is sucrose at a concentration of 7.5% ± 1.125% w/v or about 7.5% w/v. In another embodiment, the stabilizer is sucrose at a concentration of 10% ± 1.5% w/v or about 10% w/v. In another embodiment, the stabilizer is sucrose at a concentration of 12.5% ± 1.875% w/v or about 12.5% w/v. In another embodiment, the stabilizer is sucrose at a concentration of 15% ± 2.25% w/v or about 15% w/v. In another embodiment, the stabilizer is sucrose at a concentration of 20% ± 3% w/v or about 20% w/v.

[0034] In one aspect, a stable liquid pharmaceutical formulation is provided, comprising: (i) from 5 ± 0.75 mg/mL to 100 ± 15.0 mg/mL of a VEGF antagonist; (ii) from 5 ± 0.75 mg/ml to 150 ± 22.5 mg/ml of a human antibody that specifically binds to human Ang-2; (iii) from 5 mM ± 0.75 mM to 50 mM ± 7.5 mM sodium phosphate; (iv) from 0.01% ± 0.0015% to 0.1% ± 0.015% (w/v) polysorbate 20; (v) from 10 mM ± 1.5 mM to 100 mM ± 15 mM sodium chloride; and (vi) from 1% ± 0.15% to 20% ± 3% (w/v) sucrose, at a pH of from about 5.5 to about 6.5.

[0035] In one embodiment, the pharmaceutical formulation comprises (i) 40 mg/mL ± 6.0 mg/mL of aflibercept; (ii) 10 ± 1.5 mg/mL of anti-Ang-2 antibody; (iii) 10 ± 1.5 mM sodium phosphate; (iv) 0.03% ± 0.0045% (w/v) polysorbate 20; (v) 40 mM ± 6.0 mM sodium chloride; and (vi) 5% ± 0.75% (w/v) sucrose, at a pH of 6.2 ± 0.3.

[0036] In one embodiment, the pharmaceutical formulation comprises (i) 40 mg/mL ± 6.0 mg/mL of aflibercept; (ii) 20 ± 3.0 mg/mL of anti-Ang-2 antibody; (iii) 10 ± 1.5 mM sodium phosphate; (iv) 0.03% ± 0.0045% (w/v) polysorbate 20; (v) 40 mM ± 6.0 mM sodium chloride; and (vi) 5% ± 0.75% (w/v) sucrose, at a pH of 6.2 ± 0.3.

[0037] In one embodiment, the pharmaceutical formulation comprises (i) 40 mg/mL ± 6.0 mg/mL of aflibercept; (ii) 60 ± 9.0 mg/mL of anti-Ang-2 antibody; (iii) 10 ± 1.5 mM sodium phosphate; (iv) 0.03% ± 0.0045% (w/v) polysorbate 20; (v) 40 mM ± 6.0 mM sodium chloride; and (vi) 5% ± 0.75% (w/v) sucrose, at a pH of 6.2 ± 0.3.

[0038] In one embodiment, the pharmaceutical formulation comprises (i) 40 mg/mL ± 6.0 mg/mL of aflibercept; (ii) 120 ± 18.0 mg/mL of anti-Ang-2 antibody; (iii) 10 ± 1.5 mM sodium phosphate; (iv)

0.03% ± 0.0045% (w/v) polysorbate 20; (v) 40 mM ± 6.0 mM sodium chloride; and (vi) 5% ± 0.75% (w/v) sucrose, at a pH of 6.2 ± 0.3.

[0039] In one aspect, a liquid pharmaceutical formulation of the present invention is provided in a container. In one embodiment, the container is a polycarbonate vial. In another embodiment, the container is a glass vial. In one embodiment, the glass vial is a type 1 borosilicate glass vial with a fluorocarbon-coated butyl rubber stopper. In another embodiment, the container is a microinfuser. In another embodiment, the container is a syringe. In a specific embodiment, the syringe comprises a fluorocarbon-coated plunger. In one embodiment, the syringe is a 2 mL long glass syringe containing less than about 500 parts per billion of tungsten equipped with a 30-G needle, a fluorocarbon–coated butyl rubber stopper, and a latex-free, non-cytotoxic rubber tip cap. In one embodiment, the syringe is a NUOVA OMPI 2 mL long glass syringe equipped with a 30-G thin wall needle, a FLUROTEC–coated 4432/50 GRY B2-40 stopper, and a FM 27 rubber tip cap. In certain embodiments, the syringe is 1 mL, 2 mL or 3 mL plastic syringe fitted with a 27-G needle. In one embodiment, the container is a polyvinyl chloride IV bag. In another embodiment, the container is a polyolefin IV bag.

[0040] In one aspect, the present invention comprises a pre-filled syringe comprising a pharmaceutical formulation of any of the preceding aspects.

[0041] In one aspect, a kit comprising a pharmaceutical composition of any one of the preceding aspects, a container, and instructions is provided. In one embodiment, the container is a prefilled syringe. In one embodiment, the container is a borosilicate vial fitted with a FLUROTEC–coated 4432/50 rubber stopper.

[0042] Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0043] **Figure 1** shows the percent change in leakage area in rabbits with DL-alpha-aminoadipic acid (DL-alpha-AAA) – induced retinal neovascularization which have been treated with anti-Ang-2 antibody, VEGF antagonist, or a combination of anti-Ang-2 antibody and VEGF antagonist, as described in Example 2 herein.

[0044] **Figure 2** shows the percent change in leakage area in rabbits with DL-alpha-AAA – induced retinal neovascularization which have been intravitreally (IVT) treated with VEGF antagonist (VGT) (or control) and intravenously (IV) treated with anti-Ang-2 antibody (or control) according to the following therapeutic regimen, as described in Example 3 herein: (a) control IVT and control IV; (b) VGT IVT and control IV; (c) control IVT and anti-Ang-2 IV; and (d) VGT IVT and anti-Ang-2 antibody IV.

[0045] **Figure 3** shows quantification of the total subretinal lesion size (A), neovessel volume (B), and vessel density (C) of baseline, control (hFc), and anti-Ang-2 antibody (mAb1) treated groups, as described in Example 5 herein. Compared to hFc-treated controls, the mAb1- treated group showed statistically significant reduction of total lesion volume (Student t-test, p=0.0034), and a trend (26.9%) towards neovessel volume reduction (Student t-test, p=0.1658). Error bars indicate standard deviation.

DETAILED DESCRIPTION

[0046] 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.

[0047] 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.).

[0048] Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to describe in their entirety.

Methods for Treating or Ameliorating Vascular Eye Diseases or Disorders

[0049] The present invention includes methods for treating, preventing, or ameliorating at least one symptom or indication of a vascular eye disease or disorder in a subject. The methods according to this aspect of the invention comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor to the subject in need thereof. In some embodiments, the Ang-2 inhibitor is administered subcutaneously or intravenously. In some embodiments, the Ang-2 inhibitor is administered in combination with a VEGF antagonist. In some embodiments, the Ang-2 inhibitor is intravitreally administered in combination with the VEGF antagonist. In some embodiments, the Ang-2 inhibitor is administered as a single combined dosage formulation with the VEGF antagonist. In some embodiments, the Ang-2 inhibitor is administered in combination with the VEGF antagonist, wherein the Ang-2 inhibitor is administered intravenously

and the VEGF antagonist is administered intravitreally. The VEGF antagonist may be administered before, after or concurrently with the Ang-2 inhibitor.

[0050] As used herein, the terms “treat”, “treating”, or the like, mean to alleviate symptoms, eliminate the causation of symptoms either on a temporary or permanent basis, or to prevent or slow the appearance of symptoms of a neovascular eye disease. In certain embodiments, the present methods are useful for treating or ameliorating at least one symptom or indication including, but not limited to, retinal angiogenesis, neovascularization, vascular leak, retinal thickening within 500 µm of the center of the fovea, hard, yellow exudates within 500 µm of the center of the fovea with adjacent retinal thickening, and at least 1 disc area of retinal thickening, any part of which is within 1 disc diameter of the center of the fovea, blurry vision, floaters, loss of contrast, double vision, and eventual loss of vision. In the context of methods for treating a vascular eye disease such as AMD or DME, the term means that, from the initiation of treatment, the patient exhibits gain of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) letters on the Early Treatment Diabetic Retinopathy Study (EDTRS) visual acuity chart. In certain embodiments, the term means that, from initiation of treatment, vision loss of greater than or equal to 15 letters is prevented in the patient.

[0051] As used herein, the terms “prevent”, “preventing”, or the like, mean to prevent development of a symptom, indication or a complication of a vascular eye disease. In the context of methods for treating a vascular eye disease such as AMD or DME, the term means, from initiation of treatment, moderate or severe vision loss is prevented in a patient.

[0052] As used herein, a “vascular eye disease or disorder” refers to eye disease or disorders that affect blood vessels in the eye. The diseases may be caused due to abnormal angiogenesis (formation of new blood vessels) or occlusion or blockage of blood vessels. The term, as used herein, includes eye diseases or disorders associated with angiogenesis. The term includes, but is not limited to eye disease or disorder selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal neovascularization, central retinal vein occlusion, branched retinal vein occlusion, polypoidal choroidal vasculopathy, and choroidal neovascularization. In certain embodiments, the term “neovascular eye disease or disorder” may be used interchangeably with the term “eye disease or disorder associated with angiogenesis.”

[0053] In certain embodiments, the present invention includes methods for treating, preventing, or ameliorating at least one symptom or indication of an eye disease or disorder associated with angiogenesis in a subject, wherein the disease or disorder is selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal neovascularization, polypoidal choroidal vasculopathy, and choroidal neovascularization.

[0054] “Diabetic Macular Edema” (DME), as used herein, refers to a serious eye condition that affects people with diabetes (type 1 or 2). Macular edema occurs when blood vessels in the retina leak into the macula and fluid and protein deposits collect on or under the macula of the eye (a yellow central area of the retina) and causes it to thicken and swell (edema). The swelling may distort a person's central vision, as the macula is near the center of the retina at the back of the eyeball. The primary symptoms of DME include, but are not limited to, blurry vision, floaters, loss of contrast, double vision, and eventual loss of vision. The pathology of DME is characterized by breakdown of the blood-retinal barrier, normally preventing water movement in the retina, thus allowing fluid to accumulate in the retinal tissue, and presence of retinal thickening. DME is presently diagnosed during an eye examination consisting of a visual acuity test, which determines the smallest letters a person can read on a standardized chart, a dilated eye exam to check for signs of the disease, imaging tests such as optical coherence tomography (OCT) or fluorescein angiography (FA) and tonometry, an instrument that measures pressure inside the eye. The following studies are also performed to determine treatment: optical coherence tomography (OCT), fluorescein angiography, and color stereo fundus photography. DME can be broadly characterized into two main categories - Focal and Diffuse. Focal DME is characterized by specific areas of separate and distinct leakage in the macula with sufficient macular blood flow. Diffuse DME results from leakage of the entire capillary bed surrounding the macula, resulting from a breakdown of the inner blood-retina barrier of the eye. In addition to Focal and Diffuse, DME is also categorized based on clinical exam findings into clinically significant macular edema (CSME), non-CSME and CSME with central involvement (CSME-CI), which involves the fovea. The present invention includes methods to treat the above-mentioned categories of DME.

[0055] Age-related macular degeneration (AMD), as used herein, refers to a serious eye condition when the small central portion of the retina, known as the macula, deteriorates. The wet form of AMD is characterized by the growth of abnormal blood vessels from the choroid underneath the macula. This is called choroidal neovascularization. These blood vessels leak blood and fluid into the retina, causing distortion of vision that makes straight lines look wavy, as well as blind spots and loss of central vision. These abnormal blood vessels eventually scar, leading to permanent loss of central vision. The symptoms of AMD include dark, blurry areas in the center of vision; and diminished or changed color perception. AMD can be detected in a routine eye exam. One of the most common early signs of macular degeneration is the presence of drusen -- tiny yellow deposits under the retina -- or pigment clumping.

[0056] As used herein, the expression “a subject in need thereof” means a human or non-human mammal that exhibits one or more symptoms or indications of, and/or who has been diagnosed with an eye disease or disorder associated angiogenesis. The term “a subject in need thereof” may also

include, *e.g.*, subjects who, prior to treatment, exhibit (or have exhibited) one or more indications of a neovascular eye disease such as, *e.g.*, retinal angiogenesis, neovascularization, vascular leak, retinal thickening within 500 µm of the center of the fovea, hard, yellow exudates within 500 µm of the center of the fovea with adjacent retinal thickening, and at least 1 disc area of retinal thickening, any part of which is within 1 disc diameter of the center of the fovea, blurry vision, floaters, loss of contrast, double vision, and eventual loss of vision.

[0057] In the context of the invention, a "subject in need thereof" also includes human or non-human mammal who has a vascular eye disease or disorder selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal neovascularization, central retinal vein occlusion, branched retinal vein occlusion, polypoidal choroidal vasculopathy, and choroidal neovascularization.

[0058] In the context of the present invention, "a subject in need thereof" may include a subset of population which is more susceptible to DME or AMD or may show an elevated level of a DME-associated or an AMD-associated biomarker. For example, "a subject in need thereof" may include a subject suffering from diabetes for more than 10 years, have frequent high blood sugar levels or high fasting blood glucose levels. In certain embodiments, the term "a subject in need thereof" includes a subject who, prior to or at the time of administration of the Ang-2 inhibitor and/or VEGF antagonist, has or is diagnosed with diabetes. In certain embodiments, the term "a subject in need thereof" includes a subject who, prior to or at the time of administration of the Ang-2 inhibitor and/or VEGF antagonist, is more than 50 years old. In some embodiments, the term "a subject in need thereof" includes subjects who are smokers, or subjects with high blood pressure or high cholesterol.

[0059] The present invention includes methods for treating, preventing or reducing the severity of a vascular eye disease comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor in combination with a VEGF antagonist to a subject in need thereof, wherein the pharmaceutical composition is administered to the subject in multiple doses, *e.g.*, as part of a specific therapeutic dosing regimen. For example, the therapeutic dosing regimen may comprise administering multiple doses of the pharmaceutical composition to the subject at a frequency of about once a day, once every two days, once every three days, once every four days, once every five days, once every six days, once a week, once every two weeks, once every three weeks, once every four weeks, once a month, once every two months, once every three months, once every four months, or less frequently. In certain embodiments, the therapeutic dosing regimen may comprise administering multiple doses of the pharmaceutical composition to the subject at a frequency of once a day or 2 times a day or more.

[0060] The methods of the present invention, according to certain embodiments, comprise

administering to a subject a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor in combination with a VEGF antagonist. In certain embodiments, the Ang-2 inhibitor of the invention may be administered in combination with therapy including laser treatment to stop leakage into the macula. As used herein, the phrase 'in combination with" means that the pharmaceutical composition comprising an Ang-2 inhibitor is administered to the subject at the same time as, just before, or just after administration of the VEGF antagonist.

[0061] The present invention also includes methods for inhibiting or reducing or suppressing vascular leak in a subject. In certain embodiments, the methods according to this aspect of the invention comprise administering to the subject one or more doses of a pharmaceutical composition comprising an Ang-2 inhibitor to reduce or inhibit vascular leak in the eye of a subject. In certain other embodiments, the methods comprise administering to the subject one or more doses of a pharmaceutical composition comprising an Ang-2 inhibitor in combination with a VEGF antagonist to reduce or inhibit vascular leak in the eye of a subject. In certain embodiments, the vascular leak is inhibited for more than 3 weeks, more than 4 weeks, more than 8 weeks, or more than 10 weeks than in a subject who has been administered the VEGF antagonist alone.

[0062] The methods of the present invention, according to certain embodiments, comprise administering to a subject a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor in combination with a VEGF antagonist. As used herein, the phrase 'in combination with" means that the pharmaceutical composition comprising an Ang-2 inhibitor is administered to the subject at the same time as, just before, or just after administration of the VEGF antagonist. In certain embodiments, the VEGF antagonist is administered as a co-formulation with the Ang-2 inhibitor. In a related embodiment, the present invention includes methods comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor to a subject to provide a greater therapeutic effect or synergistic effect as compared to administration of the VEGF antagonist alone. The subject may be on a therapeutic regimen of intravitreally administered VEGF antagonist. In some embodiments, the Ang-2 inhibitor is added to this therapeutic regimen, wherein one or more intravitreal injections of the VEGF antagonist may be reduced or the duration between successive intravitreal injections may be increased.

[0063] In certain embodiments, the present invention provides methods to treat a vascular eye disease, the methods comprising administering one or more doses of a pharmaceutical composition comprising therapeutically effective amount of an anti-Ang-2 inhibitor and therapeutically effective amount of a VEGF antagonist to a subject in need thereof. In certain embodiments, the pharmaceutical composition is intravitreally administered to the subject. In certain embodiments, the pharmaceutical composition comprises about 10 mg/mL to about 120 mg/mL of the anti-Ang-2 inhibitor and about 40 mg/mL of the VEGF antagonist. In certain embodiments, the methods

comprise administering an initial dose of the pharmaceutical composition, followed by one or more secondary doses, wherein each secondary dose is administered 1 to 4 weeks after the immediately preceding dose. In certain embodiments, one or more tertiary doses of the pharmaceutical composition are administered, wherein each tertiary dose is administered 5 to 12 weeks after the immediately preceding dose. In certain embodiments, each dose of the pharmaceutical composition comprises about 0.5 to about 6 mg of the anti-Ang-2 inhibitor and about 2 mg of the VEGF antagonist.

[0064] In certain embodiments, the present invention provides methods to reduce the number of intravitreal injections in a subject with a vascular eye disease, the methods comprising administering a pharmaceutical composition comprising an anti-Ang-2 inhibitor and a VEGF antagonist, wherein the intravitreal administration is reduced to once in 8 weeks as compared to a subject administered with the anti-Ang-2 inhibitor or the VEGF antagonist alone.

[0065] The methods of the present invention are useful for treating or preventing vascular eye disorders in patients that have been diagnosed with or are at risk of being afflicted with a vascular eye disorder. Generally, the methods of the present invention demonstrate efficacy within 36 weeks of the initiation of the treatment regimen (with the initial dose administered at “week 0”), *e.g.*, by the end of week 6, by the end of week 12, by the end of week 18, by the end of week 24, etc. In the context of methods for treating angiogenic eye disorders such as AMD, and DME, “efficacy” means that, from the initiation of treatment, the patient exhibits a loss of 10 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.

Angiopoietin-2 (Ang-2) Inhibitors

[0066] As used herein, the term "Ang-2” or “ANG2” means a human angiopoietin-2, which is generally known as an autocrine antagonist of Tie2 activation. Ang-2 is generally known in the art to “prime” the vascular endothelium to receive the effects of cytokines. Ang-2 is strongly expressed in tumor vasculature, and is generally thought to act synergistically with other cytokines (*i.e.,* vascular endothelial growth factor) to promote angiogenesis and tumor progression.

[0067] As used herein, an “Ang-2 inhibitor” (also referred to herein as an “Ang-2 antagonist,” an “Ang-2 blocker,” etc.) is any agent which binds to or interacts with Ang-2, and inhibits or attenuates the normal biological signaling function/activity of Ang-2.

[0068] Non-limiting examples of categories of Ang-2 inhibitors include small molecule Ang-2 inhibitors, anti-Ang-2 aptamers, peptide-based Ang-2 inhibitors (*e.g.*, “peptibody” molecules), “receptor-bodies” (*e.g.*, engineered molecules comprising the receptor-binding domain of an Ang-2

component), and antibodies or antigen-binding fragments of antibodies that specifically bind human Ang-2. In certain embodiments, the Ang-2 inhibitor is an antibody or antigen binding fragment thereof as disclosed in *e.g.*, US Patent Application Publication No. US20110027286. In certain embodiments, the anti-Ang-2 antibody or antigen binding fragment thereof comprises a heavy chain variable region (HCVR) having an amino acid sequence of SEQ ID NO: 1 and a light chain variable region (LCVR) having an amino acid sequence of SEQ ID NO: 2.

[0069] The methods of the present invention comprise administering to a subject in need thereof a therapeutic composition comprising an Ang-2 inhibitor.

Anti-Ang-2 Antibodies and Antigen-Binding Fragments Thereof

[0070] According to certain exemplary embodiments of the present invention, the Ang-2 inhibitor is an anti- Ang-2 antibody or antigen-binding fragment thereof. The term "antibody," as used herein, includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). In a typical antibody, each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti- Ang-2 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0071] The term "antibody," as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries),

or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0072] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0073] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0074] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide

bond(s)).

[0075] The term "antibody," as used herein, also includes multispecific (*e.g.*, bispecific) antibodies. A multispecific antibody or antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format may be adapted for use in the context of an antibody or antigen-binding fragment of an antibody of the present invention using routine techniques available in the art. For example, the present invention includes methods comprising the use of bispecific antibodies wherein one arm of an immunoglobulin is specific for IL-4Rα or a fragment thereof, and the other arm of the immunoglobulin is specific for a second therapeutic target or is conjugated to a therapeutic moiety. Exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED) body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (*see*, *e.g.*, Klein *et al*. 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific antibodies can also be constructed using peptide/nucleic acid conjugation, *e.g.*, wherein unnatural amino acids with orthogonal chemical reactivity are used to generate site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric complexes with defined composition, valency and geometry. (*See*, *e.g.*, Kazane *et al*., *J. Am. Chem. Soc*. [*Epub*: *Dec. 4, 2012*]).

[0076] The antibodies used in the methods of the present invention may be human antibodies. The term “human antibody,” as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may nonetheless include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term “human antibody,” as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0077] The antibodies used in the methods of the present invention may be recombinant human antibodies. The term “recombinant human antibody,” as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody

library (described further below), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0078] According to certain embodiments, the antibodies used in the pharmaceutical formulations and methods of the present invention specifically bind Ang-2. The term "specifically binds," or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Methods for determining whether an antibody specifically binds to an antigen are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. For example, an antibody that "specifically binds" Ang-2, as used in the context of the present invention, includes antibodies that bind Ang-2or portion thereof with a K_D of less than about 500 nM, less than about 300 nM, less than about 200 nM, less than about 100 nM, less than about 90 nM, less than about 80 nM, less than about 70 nM, less than about 60 nM, less than about 50 nM, less than about 40 nM, less than about 30 nM, less than about 20 nM, less than about 10 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM or less than about 0.5 nM, as measured in a surface plasmon resonance assay. An isolated antibody that specifically binds human Ang-2 may, however, have cross-reactivity to other antigens, such as Ang-2 molecules from other (non-human) species.

[0079] According to certain exemplary embodiments of the present invention, the Ang-2 inhibitor is an anti-Ang-2 antibody, or antigen-binding fragment thereof comprising a heavy chain variable region (HCVR), light chain variable region (LCVR), and/or complementarity determining regions (CDRs) comprising any of the amino acid sequences of the anti- Ang-2 antibodies as set forth in US Patent Application Publication No. US20110027286.

[0080] In certain exemplary embodiments, the anti-Ang-2 antibody or antigen-binding fragment thereof that can be used in the context of the methods of the present invention comprises the heavy chain complementarity determining regions (HCDRs) of a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 1 and the light chain complementarity

determining regions (LCDRs) of a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 2. According to certain embodiments, the anti- Ang-2 antibody or antigen-binding fragment thereof comprises three HCDRs (HCDR1, HCDR2 and HCDR3) and three LCDRs (LCDR1, LCDR2 and LCDR3), wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 3; the HCDR2 comprises the amino acid sequence of SEQ ID NO: 4; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 5; the LCDR1 comprises the amino acid sequence of SEQ ID NO: 6; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 7; and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 8. In yet other embodiments, the anti- Ang-2 antibody or antigen-binding fragment thereof comprises an HCVR comprising SEQ ID NO: 1 and an LCVR comprising SEQ ID NO: 2. In certain embodiments, the methods of the present invention comprise the use of an anti- Ang-2 antibody, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the anti-Ang-2 antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 10. An exemplary antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 9 and a light chain comprising the amino acid sequence of SEQ ID NO: 10 is the fully human anti-Ang-2 antibody known as nesvacumab. According to certain exemplary embodiments, the methods of the present invention comprise the use of nesvacumab, or a bioequivalent thereof. The term “bioequivalent”, as used herein, refers to anti- Ang-2 antibodies or Ang-2-binding proteins or fragments thereof that are pharmaceutical equivalents or pharmaceutical alternatives whose rate and/or extent of absorption do not show a significant difference with that of nesvacumab when administered at the same molar dose under similar experimental conditions, either single dose or multiple dose. In the context of the invention, the term refers to antigen-binding proteins that bind to Ang-2 which do not have clinically meaningful differences with nesvacumab in their safety, purity and/or potency.

[0081] The non-limiting, exemplary antibody used in the Examples herein is referred to as "H1H685P”, as in US 2011/0027286. This antibody comprises an HCVR/LCVR amino acid sequence pair having SEQ ID NOs: 1/2, and HCDR1-HCDR2-HCDR3 / LCDR1-LCDR2-LCDR3 domains represented by SEQ ID NOs: 3 – 4 – 5 / SEQ ID NOs: 6 – 7 – 8.

[0082] Other antibodies to human Ang-2 are described in patent application publications US 2010/0166768, US 2011/0065902, and WO 2010/077854, which are herein incorporated by reference.

[0083] The amount of antibody, or antigen-binding fragment thereof, contained within the pharmaceutical formulations of the present invention may vary depending on the specific properties desired of the formulations, as well as the particular circumstances and purposes for which the formulations are intended to be used. In certain embodiments, the pharmaceutical formulations are

liquid formulations that may contain 5 ± 0.75 mg/mL to 150 ± 22.5 mg/mL of antibody; 7.5 ± 1.125 mg/mL to 140 ± 21 mg/mL of antibody. For example, the formulations of the present invention may comprise about 10 mg/mL; about 20 mg/mL; about 30 mg/mL; about 50 mg/mL; about 60 mg/mL; about 80 mg/mL; about 100 mg/mL; about 120 mg/mL; or about 150 mg/mL of an antibody or an antigen-binding fragment thereof, that binds specifically to human Ang-2.

VEGF antagonists

[0084] As used herein, a "VEGF antagonist" is any agent that binds to or interacts with VEGF, inhibits the binding of VEGF to its receptors (VEGFR1 and VEGFR2), and/or inhibits the biological signaling and activity of VEGF. 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 (*e.g.*, ranibizumab [LUCENTIS®]), anti-VEGF receptor antibodies (*e.g.*, anti-VEGFR1 antibodies, anti-VEGFR2 antibodies, etc.), small molecule inhibitors of VEGF (*e.g.*, sunitinib), and VEGF receptor-based chimeric molecules or VEGF-inhibiting fusion proteins (also referred to herein as "VEGF-Traps").

[0085] 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) (also known as aflibercept; marketed under the product name EYLEA®). In certain embodiments, aflibercept is encoded by the amino acid sequence of SEQ ID NO: 11.

[0086] The amount of the VEGF antagonist contained within the pharmaceutical formulations of the present invention may vary depending on the specific properties desired of the formulations, as well as the particular circumstances and purposes for which the formulations are intended to be used. In certain embodiments, the pharmaceutical formulations are liquid formulations that may contain 5 ± 0.75 mg/mL to 150 ± 22.5 mg/mL of VEGF antagonist; 10 ± 1.5 mg/mL to 100 ± 15.0 mg/mL of VEGF antagonist; 20 ± 3 mg/mL to 80 ± 12 mg/mL of VEGF antagonist; 30 ± 4.5 mg/mL to 70 ± 10.5 mg/mL of VEGF antagonist or 40 ± 6.0 mg/mL of the VEGF antagonist. For example, the formulations of the present invention may comprise about 20 mg/mL; about 30 mg/mL; about 40 mg/mL; about 50 mg/mL; or about 60 mg/mL of a VEGF antagonist.

Combination Therapies

[0087] The methods of the present invention, according to certain embodiments, comprise administering to the subject a VEGF antagonist in combination with an anti-Ang-2 antibody. As used herein, the expression "in combination with" means that the VEGF antagonist is administered before, after, or concurrent with the pharmaceutical composition comprising the anti-Ang-2 antibody. The term “in combination with” also includes sequential or concomitant administration of anti-Ang-2 antibody and a VEGF antagonist. For example, when administered "before" the pharmaceutical composition comprising the anti-Ang-2 antibody, the VEGF antagonist may be administered more than 72 hours, about 72 hours, about 60 hours, about 48 hours, about 36 hours, about 24 hours, about 12 hours, about 10 hours, about 8 hours, about 6 hours, about 4 hours, about 2 hours, about 1 hour, about 30 minutes, about 15 minutes or about 10 minutes prior to the administration of the pharmaceutical composition comprising the anti-Ang-2 antibody. When administered "after" the pharmaceutical composition comprising the anti-Ang-2 antibody, the VEGF antagonist may be administered about 10 minutes, about 15 minutes, about 30 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 60 hours, about 72 hours, or more than 72 hours after the administration of the pharmaceutical composition comprising the anti-Ang-2 antibody. Administration "concurrent" with the pharmaceutical composition comprising the anti-Ang-2 antibody means that the VEGF antagonist is administered to the subject in a separate dosage form within less than 5 minutes (before, after, or at the same time) of administration of the pharmaceutical composition comprising the anti-Ang-2 antibody, or administered to the subject as a single combined dosage formulation comprising both the VEGF antagonist and the anti-Ang-2 antibody.

[0088] Combination therapies may include an anti-Ang-2 antibody of the invention and a VEGF antagonist (*e.g.*, aflibercept, a VEGF-Trap, *see*, *e.g.*, US 7,087,411 (also referred to herein as a "VEGF-inhibiting fusion protein"), anti-VEGF antibody (*e.g.*, ranibizumab), a small molecule kinase inhibitor of VEGF receptor (*e.g.*, sunitinib, sorafenib or pazopanib), etc.

[0089] The methods of the invention comprise administering an anti-Ang-2 antibody in combination with a VEGF antagonist for additive or synergistic activity to treat or ameliorate at least one symptom or indication of an eye disease or disorder selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal neovascularization, central retinal vein occlusion, branched retinal vein occlusion, polypoidal choroidal vasculopathy, and choroidal neovascularization.

Pharmaceutical Compositions and Formulations

[0090] The present invention includes methods which comprise administering an Ang-2 inhibitor

to a subject wherein the Ang-2 inhibitor is contained within a pharmaceutical composition. In certain embodiments, the pharmaceutical composition further comprises a VEGF antagonist. In alternate embodiments, the Ang-2 inhibitor and the VEGF antagonist may be in own separate pharmaceutical dosage formulation. The pharmaceutical compositions of the invention may be formulated with suitable carriers, excipients, and other agents that provide suitable transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0091] As used herein, the expression "pharmaceutical formulation" means a combination of at least one active ingredient (*e.g.*, a small molecule, macromolecule, compound, etc. which is capable of exerting a biological effect in a human or non-human animal), and at least one inactive ingredient which, when combined with the active ingredient or one or more additional inactive ingredients, is suitable for therapeutic administration to a human or non-human animal. The term "formulation", as used herein, means "pharmaceutical formulation" unless specifically indicated otherwise. The present invention provides pharmaceutical formulations comprising at least one therapeutic polypeptide. According to certain embodiments of the present invention, the therapeutic polypeptide is an antibody, or an antigen-binding fragment thereof, which binds specifically to human angiopoietin-2 (Ang-2) protein. According to certain other embodiments, the present invention provides pharmaceutical formulations comprising more than one therapeutic polypeptide. More specifically, the present invention includes pharmaceutical formulations that comprise: (i) a human antibody that specifically binds to human Ang-2; (ii) a VEGF antagonist; (iii) a sodium phosphate buffer; (iv) an organic co-solvent that is a non-ionic surfactant; (v) a tonicity agent such as sodium chloride; and (vi) a thermal stabilizer that is a carbohydrate. Specific exemplary components and formulations included within the present invention are described in detail below.

[0092] The amount of antibody, or antigen-binding fragment thereof, contained within the pharmaceutical formulations of the present invention may vary depending on the specific properties desired of the formulations, as well as the particular circumstances and purposes for which the formulations are intended to be used. In certain embodiments, the pharmaceutical formulations are liquid formulations that may contain 5 ± 0.75 mg/mL to 150 ± 22.5 mg/mL of antibody; 7.5 ± 1.125

mg/mL to 140 ± 21 mg/mL of antibody; 10 ± 1.5 mg/mL to 130 ± 19.5 mg/mL of antibody; 10 ± 1.5 mg/mL of antibody; 20 ± 3 mg/mL of antibody; 60 ± 9 mg/mL of antibody; or 120 ± 18 mg/mL of antibody. For example, the formulations of the present invention may comprise about 10 mg/mL; about 20 mg/mL; about 40 mg/mL; about 60 mg/mL; about 80 mg/mL; about 100 mg/mL; about 120 mg/mL; or about 140 mg/mL of an antibody or an antigen-binding fragment thereof that binds specifically to human Ang-2.

[0093] In certain embodiments, the pharmaceutical formulations are liquid formulations that may contain 5 ± 0.75 mg/mL to 100 ± 15 mg/mL of a VEGF antagonist. For example, the formulations of the present invention may comprise about 5 mg/mL; about 10 mg/mL; about 15 mg/mL; about 20 mg/mL; about 25 mg/mL; about 30 mg/mL; about 35 mg/mL; about 40 mg/mL; about 50 mg/mL; about 60 mg/mL; about 70 mg/mL; about 80 mg/mL; about 90 mg/mL; or about 100 mg/mL of a VEGF antagonist such as aflibercept.

[0094] In certain embodiments, the pharmaceutical formulations are stable liquid co-formulations comprising about 5 mg/mL to about 150 mg/mL of the anti-Ang-2 antibody and about 5 to 100 mg/mL of the VEGF antagonist.

[0095] The pharmaceutical formulations of the present invention comprise one or more excipients. The term "excipient", as used herein, means any non-therapeutic agent added to the formulation to provide a desired consistency, viscosity or stabilizing effect.

[0096] In certain embodiments, the pharmaceutical formulation of the invention comprises at least one organic cosolvent in a type and in an amount that stabilizes the human Ang-2 antibody under conditions of rough handling or agitation, such as, *e.g.,* vortexing. In some embodiments, what is meant by "stabilizes" is the prevention of the formation of more than 4% aggregated antibody of the total amount of antibody (on a molar basis) over the course of rough handling. In some embodiments, rough handling is vortexing a solution containing the antibody and the organic cosolvent for about 60 minutes or about 120 minutes.

[0097] In certain embodiments, the organic cosolvent is a non-ionic surfactant, such as an alkyl poly(ethylene oxide). Specific non-ionic surfactants that can be included in the formulations of the present invention include, *e.g.*, polysorbates such as polysorbate 20, polysorbate 28, polysorbate 40, polysorbate 60, polysorbate 65, polysorbate 80, polysorbate 81, and polysorbate 85; poloxamers such as poloxamer 181, poloxamer 188, poloxamer 407; or polyethylene glycol (PEG). Polysorbate 20 is also known as TWEEN 20, sorbitan monolaurate and polyoxyethylenesorbitan monolaurate. Poloxamer 188 is also known as PLURONIC F68.

[0098] The amount of non-ionic surfactant contained within the pharmaceutical formulations of the present invention may vary depending on the specific properties desired of the formulations, as well as the particular circumstances and purposes for which the formulations are intended to be used.

In certain embodiments, the formulations may contain 0.01% ± 0.0015% to 1% ± 0.15% surfactant. For example, the formulations of the present invention may comprise about 0.0085%; about 0.01%; about 0.02%; about 0.03%; about 0.04%; about 0.05%; about 0.06%; about 0.07%; about 0.08%; about 0.09%; about 0.1%; about 0.11%; about 0.12%; about 0.13%; about 0.14%; about 0.15%; about 0.16%; about 0.17%; about 0.18%; about 0.19%; about 0.20%; about 0.21%; about 0.22%; about 0.23%; about 0.24%; about 0.25%; about 0.3%; about 0.4%; about 0.5%; about 0.6%; about 0.7%; about 0.8%; about 0.9%; about 1%; about 1.1%; about 1.15%; or about 1.2% polysorbate 20, polysorbate 80 or poloxamer 188.

[0099] The pharmaceutical formulations of the present invention may also comprise one or more stabilizers in a type and in an amount that stabilizes the human Ang-2 antibody under conditions of thermal stress. In some embodiments, what is meant by "stabilizes" is maintaining greater than about 93% of the antibody in a native conformation when the solution containing the antibody and the thermal stabilizer is kept at about 45°C for up to about 28 days. In some embodiments, what is meant by "stabilizes" is wherein less than about 4% of the antibody is aggregated when the solution containing the antibody and the thermal stabilizer is kept at about 45°C for up to about 28 days. In some embodiments, what is meant by "stabilizes" is maintaining greater than about 96% of the antibody in a native conformation when the solution containing the antibody and the thermal stabilizer is kept at about 37°C for up to about 28 days. In some embodiments, what is meant by "stabilizes" is wherein less than about 2% of the antibody is aggregated when the solution containing the antibody and the thermal stabilizer is kept at about 37°C for up to about 28 days. As used herein, "native" means the major form of the antibody by size exclusion, which is generally an intact monomer of the antibody.

[00100] In certain embodiments, the thermal stabilizer is a sugar or sugar alcohol selected from sucrose, sorbitol, glycerol, trehalose and mannitol, or any combination thereof, the amount of which contained within the formulation can vary depending on the specific circumstances and intended purposes for which the formulation is used. In certain embodiments, the formulations may contain about 1% to about 20% sugar or sugar alcohol; about 2% to about 18% sugar or sugar alcohol; about 3 % to about 15% sugar or sugar alcohol; about 4% to about 10% sugar or sugar alcohol; or about 5% sugar or sugar alcohol. For example, the pharmaceutical formulations of the present invention may comprise 4%± 0.6%; 5% ± 0.75%; 6% ± 0.9%; 7% ± 1.05%; 8% ± 1.2%; 9% ± 1.35%; 10% ± 1.5%; 11% ± 1.65%; 12% ± 1.8%; 13% ± 1.95%; or about 14% ± 2.1% sugar or sugar alcohol (*e.g.,* sucrose, trehalose or mannitol).

[00101] In certain embodiments, the pharmaceutical formulations of the present invention comprise a tonicity agent such as sodium chloride or potassium chloride. In some embodiments, the tonicity

agent is sodium chloride. In some embodiments, the the sodium chloride is present at a concentration of 5 mM ± 0.75 mM to 100 mM ± 15.0 mM; 10 mM ± 1.5 mM to 50 mM ± 7.5 mM; 40 mM ± 6.0 mM; or about 40 mM.

[00102] The pharmaceutical formulations of the present invention may also comprise a buffer or buffer system, which serves to maintain a stable pH and to help stabilize the human Ang-2 antibody. In some embodiments, what is meant by "stabilizes" is wherein less than 5% ± 0.5% or no more than about 4.3% of the antibody is aggregated when the solution containing the antibody and the buffer is kept at about 45°C for up to about 28 days. In some embodiments, what is meant by "stabilizes" is wherein at least 92% ± 0.5% of the antibody is in its native conformation as determined by size exclusion chromatography when the solution containing the antibody and the buffer is kept at about 45°C for up to about 28 days. By "native" or "native conformation", what is meant is the antibody fraction that is not aggregated or degraded. This is generally determined by an assay that measures the relative size of the antibody entity, such as a size exclusion chromatographic assay. The non-aggregated and non-degraded antibody elutes at a fraction that equates to the native antibody, and is generally the main elution fraction. Aggregated antibody elutes at a fraction that indicates a size greater than the native antibody. Degraded antibody elutes at a fraction that indicates a size less than the native antibody.

[00103] In some embodiments, what is meant by "stabilizes" is wherein at least 52% ± 0.5% of the antibody is in its main charge form as determined by cation exchange chromatography when the solution containing the antibody and the buffer is kept at about 45°C for up to about 28 days. By "main charge" or "main charge form", what is meant is the fraction of antibody that elutes from an ion exchange resin in the main peak, which is generally flanked by more "basic" peaks on one side and more "acidic" peaks on the other side.

[00104] The pharmaceutical formulations of the present invention may have a pH of from about 5.5 to about 6.5. For example, the formulations of the present invention may have a pH of about 5.5; about 5.6; about 5.7; about 5.8; about 5.9; about 6.0; about 6.1; about 6.2; about 6.3; about 6.4; or about 6.5. In some embodiments, the pH is 6.2 ± 0.3; 6.2 ±0.2; 6.2± 0.1; about 6.2; or 6.2.

[00105] In some embodiments, the buffer or buffer system comprises at least one buffer that has a buffering range that overlaps fully or in part the range of pH 5.5 – 7.4. In one embodiment, the buffer has a pKa of about 6.2 ± 0.5. In certain embodiments, the buffer comprises a sodium phosphate buffer. In certain embodiments, the sodium phosphate is present at a concentration of 5 mM ± 0.75 mM to 15 mM ± 2.25 mM; 6 mM ± 0.9 mM to 14 mM ± 2.1 mM; 7 mM ± 1.05 mM to 13 mM ± 1.95 mM; 8 mM ± 1.2 mM to 12 mM ± 1.8 mM; 9 mM ± 1.35 mM to 11 mM ± 1.65 mM; 10 mM ± 1.5 mM; or about 10 mM. In certain embodiments, the buffer system comprises sodium

phosphate at 10 mM ± 1.5 mM, at a pH of 6.2 ± 0.3 or 6.1 ± 0.3.

[00106] Exemplary formulations comprising a VEGF antagonist that can be used in the context of the present invention are disclosed, *e.g.*, in US Patents 7,531,173 and 7,608,261. Exemplary pharmaceutical compositions comprising an anti-Ang-2 antibody that can be used in the context of the present invention are disclosed, *e.g.*, in US Patent Application Publication No. 20130186797.

Stability of the Pharmaceutical Formulations

[00107] The pharmaceutical formulations of the present invention typically exhibit high levels of stability. The term "stable", as used herein in reference to the pharmaceutical formulations, means that the antibodies within the pharmaceutical formulations retain an acceptable degree of chemical structure or biological function after storage under defined conditions. A formulation may be stable even though the antibody contained therein does not maintain 100% of its chemical structure or biological function after storage for a defined amount of time. Under certain circumstances, maintenance of about 90%, about 95%, about 96%, about 97%, about 98% or about 99% of an antibody's structure or function after storage for a defined amount of time may be regarded as "stable".

[00108] Stability can be measured, *inter alia*, by determining the percentage of native antibody that remains in the formulation after storage for a defined amount of time at a defined temperature. The percentage of native antibody can be determined by, *inter alia*, size exclusion chromatography (*e.g.*, size exclusion high or ultra performance liquid chromatography [SE-HPLC or SE-UPLC]), such that native means non-aggregated and non-degraded. An "acceptable degree of stability", as that phrase is used herein, means that at least 90% of the native form of the antibody can be detected in the formulation after storage for a defined amount of time at a given temperature. In certain embodiments, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the native form of the antibody can be detected in the formulation after storage for a defined amount of time at a defined temperature. The defined amount of time after which stability is measured can be at least 14 days, at least 28 days, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least 18 months, at least 24 months, or more. The defined temperature at which the pharmaceutical formulation may be stored when assessing stability can be any temperature from about -80ºC to about 45ºC, *e.g.*, storage at about -80ºC, about -30ºC, about -20ºC, about 0ºC, about 4º-8°C, about 5ºC, about 25ºC, about 35ºC, about 37ºC, or about 45ºC. For example, a pharmaceutical formulation may be deemed stable if after nine months of storage at 5ºC, greater than about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC or SE-

UPLC. A pharmaceutical formulation may also be deemed stable if after six months of storage at 25ºC, greater than about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC or SE-UPLC. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 37ºC, greater than about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC or SE-UPLC. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 45ºC, greater than about 93%, 94%, 95%, 96%, 97%, 98% or 99% of native antibody is detected by SE-HPLC or SE-UPLC. A pharmaceutical formulation may also be deemed stable if after six months of storage at -20ºC, greater than about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may also be deemed stable if after six months of storage at -30ºC, greater than about greater than about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC or SE-UPLC. A pharmaceutical formulation may also be deemed stable if after six months of storage at -80ºC, greater than about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC or SE-UPLC.

[00109] Stability can be measured, *inter alia*, by determining the percentage of antibody that forms in an aggregate within the formulation after storage for a defined amount of time at a defined temperature, wherein stability is inversely proportional to the percent aggregate that is formed. The percentage of aggregated antibody can be determined by, *inter alia*, size exclusion chromatography (*e.g.*, size exclusion high performance liquid chromatography [SE-HPLC]). An "acceptable degree of stability", as that phrase is used herein, means that at most 6% of the antibody is in an aggregated form detected in the formulation after storage for a defined amount of time at a given temperature. In certain embodiments an acceptable degree of stability means that at most about 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% of the antibody can be detected in an aggregate in the formulation after storage for a defined amount of time at a given temperature. The defined amount of time after which stability is measured can be at least 2 weeks, at least 28 days, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least 18 months, at least 24 months, or more. The temperature at which the pharmaceutical formulation may be stored when assessing stability can be any temperature from about -80ºC to about 45ºC, *e.g.*, storage at about -80ºC, about -30ºC, about -20ºC, about 0ºC, about 4º-8°C, about 5ºC, about 25ºC, about 35ºC, about 37ºC or about 45ºC. For example, a pharmaceutical formulation may be deemed stable if after nine months of storage at 5ºC, less than about 2%, 1.75%, 1.5%, 1.25%, 1%, 0.75%, 0.5%, 0.25%, or 0.1% of the antibody is detected in an aggregated form. A pharmaceutical formulation may also be deemed stable if after six months of storage at 25ºC, less than about 2%, 1.75%, 1.5%, 1.25%, 1%, 0.75%, 0.5%, 0.25%, or 0.1% of the

antibody is detected in an aggregated form. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 45ºC, less than about 4%, 3.5%, 3%, 2.5%, 2%, 1.5%, 1%, 0.5%, or 0.1% of the antibody is detected in an aggregated form. A pharmaceutical formulation may also be deemed stable if after three months of storage at -20ºC, -30ºC, or -80ºC less than about 2%, 1.9%, 1.8%, 1.7%, 1.6%, 1.5%, 1%, 0.5%, or 0.1% of the antibody is detected in an aggregated form.

[00110] Stability can be measured, *inter alia*, by determining the percentage of antibody that migrates in a more acidic fraction during ion exchange ("acidic form") than in the main fraction of antibody ("main charge form"), wherein stability is inversely proportional to the fraction of antibody in the acidic form. While not wishing to be bound by theory, deamidation of the antibody may cause the antibody to become more negatively charged and thus more acidic relative to the non-deamidated antibody (see, *e.g.,* Robinson, N., Protein Deamidation, *PNAS*, April 16, 2002, 99(8):5283-5288). The percentage of "acidified" antibody can be determined by ion exchange chromatography (*e.g.*, cation exchange high performance liquid chromatography [CEX-HPLC]). An "acceptable degree of stability", as that phrase is used herein, means that at most 52% of the antibody is in a more acidic form detected in the formulation after storage for a defined amount of time at a defined temperature. In certain embodiments an acceptable degree of stability means that at most about 52%, 50%, 45%, 40%, 35%, 30%, 29%, 28%, 27%, 26%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% of the antibody can be detected in an acidic form in the formulation after storage for a defined amount of time at a given temperature. The defined amount of time after which stability is measured can be at least 2 weeks, at least 28 days, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least 18 months, at least 24 months, or more. The temperature at which the pharmaceutical formulation may be stored when assessing stability can be any temperature from about -80ºC to about 45ºC, *e.g.*, storage at about -80ºC, about -30ºC, about -20ºC, about 0ºC, about 4º-8°C, about 5ºC, about 25ºC, or about 45ºC. For example, a pharmaceutical formulation may be deemed stable if after three months of storage at -80ºC, -30ºC, or -20ºC less than about 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody is in a more acidic form. A pharmaceutical formulation may also be deemed stable if after nine months of storage at 5ºC, less than about 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody is in a more acidic form. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 25ºC, less than about 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%,

22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody is in a more acidic form. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 37ºC, less than about 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody is in a more acidic form. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 45ºC, less than about 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody can be detected in a more acidic form.

[00111] Measuring the binding affinity of the antibody to its target may also be used to assess stability. For example, a formulation of the present invention may be regarded as stable if, after storage at *e.g.*, -80ºC, -30ºC, -20ºC, 5ºC, 25ºC, 37ºC, 45ºC, etc. for a defined amount of time (*e.g.*, 14 days to 6 months), the anti-Ang-2 antibody contained within the formulation binds to Ang-2 with an affinity that is at least 84%, 90%, 95%, or more of the binding affinity of the antibody prior to said storage. Binding affinity may be determined by any method, such as *e.g.,* ELISA or plasmon resonance. Biological activity may be determined by an Ang-2 activity assay, such as by contacting a cell that expresses Ang-2 with the formulation comprising the anti Ang-2 antibody. The binding of the antibody to such a cell may be measured directly, such as via FACS analysis. Alternatively, the downstream activity of the Ang-2 system may be measured in the presence of the antibody, and compared to the activity of the Ang-2 system in the absence of antibody. In some embodiments, the Ang-2 may be endogenous to the cell. In other embodiments, the Ang-2 may be ectopically expressed (*i.e.,* heterologous expression) in the cell.

[00112] Additional methods for assessing the stability of an antibody in formulation are demonstrated in the Examples presented below.

Containers and Methods of Administration

[00113] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, micro-particles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu et al., 1987, J. Biol. Chem. 262: 4429-4432). Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition 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.

[00114] For the treatment of eye disorders, the pharmaceutical formulations of the invention may be administered, *e.g.*, by eye drops, subconjunctival injection, subconjunctival implant, intravitreal injection, intravitreal implant, sub-Tenon's injection or sub-Tenon's implant.

[00115] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[00116] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[00117] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by known methods. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above 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 are 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 is preferably filled in an appropriate ampoule.

[00118] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc.

[00119] The pharmaceutical formulations of the present invention may be contained within any container suitable for storage or administration of medicines and other therapeutic compositions. For example, the pharmaceutical formulations may be contained within a sealed and sterilized plastic or glass container having a defined volume such as a vial, ampule, syringe, cartridge, bottle, or IV bag. Different types of vials can be used to contain the formulations of the present invention including, *e.g.,* clear and opaque (*e.g.*, amber) glass or plastic vials. Likewise, any type of syringe can be used to contain or administer the pharmaceutical formulations of the present invention.

[00120] The pharmaceutical formulations of the present invention may be contained within "normal tungsten" syringes or "low tungsten" syringes. As will be appreciated by persons of ordinary skill in the art, the process of making glass syringes generally involves the use of a hot tungsten rod which functions to pierce the glass thereby creating a hole from which liquids can be drawn and expelled from the syringe. This process results in the deposition of trace amounts of tungsten on the interior surface of the syringe. Subsequent washing and other processing steps can be used to reduce the amount of tungsten in the syringe. As used herein, the term "normal tungsten" means that the syringe contains greater than or equal to 500 parts per billion (ppb) of tungsten. The term "low tungsten" means that the syringe contains less than 500 ppb of tungsten. For example, a low tungsten syringe, according to the present invention, can contain less than about 490, 480, 470, 460, 450, 440, 430, 420, 410, 390, 350, 300, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10 or fewer ppb of tungsten.

[00121] The rubber plungers used in syringes, and the rubber stoppers used to close the openings of vials, may be coated to prevent contamination of the medicinal contents of the syringe or vial, or to preserve their stability. Thus, pharmaceutical formulations of the present invention, according to certain embodiments, may be contained within a syringe that comprises a coated plunger, or within a vial that is sealed with a coated rubber stopper. For example, the plunger or stopper may be coated with a fluorocarbon film. Examples of coated stoppers or plungers suitable for use with vials and syringes containing the pharmaceutical formulations of the present invention are mentioned in, *e.g.*, U.S. Patent Nos. 4,997,423; 5,908,686; 6,286,699; 6,645,635; and 7,226,554, the contents of which are incorporated by reference herein in their entireties. Particular exemplary coated rubber stoppers and plungers that can be used in the context of the present invention are commercially

available under the tradename "FluroTec®", available from West Pharmaceutical Services, Inc. (Lionville, PA). FluroTec® is an example of a flurocarbon coating used to minimize or prevent drug product from adhering to the rubber surfaces.

[00122] According to certain embodiments of the present invention, the pharmaceutical formulations may be contained within a low tungsten syringe that comprises a fluorocarbon-coated plunger.

[00123] The pharmaceutical formulations can be administered to a patient by parenteral routes such as injection (*e.g.*, subcutaneous, intravenous, intramuscular, intraperitoneal, etc.) or percutaneous, mucosal, nasal, pulmonary or oral administration. Numerous reusable pen or autoinjector delivery devices can be used to subcutaneously deliver the pharmaceutical formulations of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany). Examples of disposable pen or autoinjector delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park, IL).

[00124] The use of a microinfusor to deliver the pharmaceutical formulations of the present invention is also contemplated herein. As used herein, the term "microinfusor" means a subcutaneous delivery device designed to slowly administer large volumes (*e.g.*, up to about 2.5 mL or more) of a therapeutic formulation over a prolonged period of time (*e.g.*, about 10, 15, 20, 25, 30 or more minutes). *See*, *e.g.*, U.S. 6,629,949; US 6,659,982; and Meehan *et al.*, *J. Controlled Release 46*:107-116 (1996). Microinfusors are particularly useful for the delivery of large doses of therapeutic proteins contained within high concentration (*e.g.*, about 100, 125, 150, 175, 200 or more mg/mL) or viscous solutions.

[00125] In one embodiment, the pharmaceutical formulation is administered via an IV drip, such that the formulation is diluted in an IV bag containing a physiologically acceptable solution. In one embodiment, pharmaceutical composition is a compounded sterile preparation in an intravenous infusion bag, such that a single dose of drug product is diluted into 100 mL, 250 mL (or other like amount suitable for intravenous drip delivery) of a physiological buffer (e.g., 0.9% saline). In some

embodiments, the infusion bag is made of a polyvinyl chloride (e.g., VIAFLEX, Baxter, Deerfield, Illinois). In some embodiments, the infusion bag is made of a polyolefin (EXCEL IV Bags, Braun Medical Inc., Bethlehem, Pennsylvania).

[00126] In certain embodiments, the liquid formulation comprising of from 10 mg/mL to 120 mg/mL of anti-Ang-2 antibody is comprised in a prefilled syringe and is administered intravitreally in a volume of approximately upto 100 µL. In certain embodiments, the liquid formulation comprising of from 10 mg/mL to 120 mg/mL of anti-Ang-2 antibody and from 10 mg/mL to 100 mg/mL of aflibercept is comprised in a prefilled syringe and is administered intravitreally in a volume of approximately upto 100 µL. In certain embodiments, the liquid formulation comprising of from 10 mg/mL to 120 mg/mL of anti-Ang-2 antibody and from 10 mg/mL to 100 mg/mL of aflibercept is in a prefilled syringe and is administered intravitreally in a volume of approximately upto 500 µL. In certain embodiments, the liquid formulation comprising of from 60 mg/mL to 120 mg/mL of anti-Ang-2 antibody and about 40 mg/mL of aflibercept is in a prefilled syringe and is administered intravitreally in a volume of approximately upto 500 µL. In one embodiment, the syringe is a 2 mL long glass syringe fitted with a 30-gauge thin wall needle, a fluorocarbon coated rubber plunger and a rubber needle shield. In one embodiment, the syringe is a 1 mL long glass syringe fitted with a 30-gauge thin wall needle, a fluorocarbon coated rubber plunger and a rubber needle shield.

Administration Regimens

[00127] The present invention includes methods comprising administering to a subject a pharmaceutical composition comprising an anti-Ang-2 antibody at a dosing frequency of about four times a week, twice a week, once a week, once every two weeks, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every eight weeks, once every twelve weeks, or less frequently so long as a therapeutic response is achieved. In certain embodiments, the methods involve the administration of a pharmaceutical composition comprising an anti-Ang-2 antibody in combination with a VEGF antagonist at a dosing frequency of about four times a week, twice a week, once a week, once every two weeks, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every eight weeks, once every nine weeks, once every twelve weeks, or less frequently so long as a therapeutic response is achieved.

[00128] According to certain embodiments of the present invention, multiple doses of an anti-Ang-2 antibody may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an anti-Ang-2 antibody. As used herein, "sequentially administering" means that each dose of anti-Ang-2 antibody is administered to the subject 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 an anti-Ang-2 antibody, followed by one or more secondary doses of the anti-Ang-2 antibody, and optionally followed by one or more tertiary doses of the anti-Ang-2 antibody.

[00129] According to certain embodiments of the present invention, multiple doses of a co-formulation comprising an anti-Ang-2 antibody and a VEGF antagonist may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of a co-formulation comprising an anti-Ang-2 antibody and a VEGF antagonist. As used herein, "sequentially administering" means that each dose of the anti-Ang-2 antibody in combination with the VEGF antagonist is administered to the subject 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 co-formulation comprising an anti-Ang-2 antibody and a VEGF antagonist, followed by one or more secondary doses of the co-formulated anti-Ang-2 antibody and VEGF antagonist, and optionally followed by one or more tertiary doses of the co-formulated anti-Ang-2 antibody and VEGF antagonist.

[00130] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration. 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 anti-Ang-2 antibody (or a co-formulation comprising anti-Ang-2 antibody and VEGF antagonist), but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, one or more (*e.g.*, 1, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses"). For example, an anti-Ang-2 antibody (or a co-formulation comprising anti-Ang-2 antibody and VEGF antagonist) may be administered to a patient with an eye disease or disorder at a loading dose of about 6mg followed by one or more maintenance doses of about 0.5mg to about 10mg.

[00131] In one exemplary embodiment of the present invention, each secondary and/or tertiary dose is administered 1 to 14 (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 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 anti-Ang-2 antibody (or a co-formulation comprising anti-Ang-2 antibody and VEGF antagonist) which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[00132] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an anti-Ang-2 antibody (or a co-formulation comprising anti-Ang-2 antibody and VEGF antagonist). For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[00133] 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 1 to 2 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 2 to 4 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. 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.

[00134] The present invention includes methods comprising sequential administration of an anti-Ang-2 antibody in combination with a VEGF antagonist, to a patient to treat DME or AMD. In some embodiments, the present methods comprise administering one or more doses of an anti-Ang-2 antibody followed by one or more doses of a VEGF antagonist. In certain embodiments, the present methods comprise administering a single dose of a VEGF antagonist followed by one or more doses of an anti-Ang-2 antibody. In some embodiments, one or more doses of about 0.05 mg to about 2 mg of a VEGF antagonist may be administered followed by one or more doses of about 0.05 mg to about 10 mg of the Ang-2 inhibitor. In one embodiment, one or more doses of about 1mg/kg to about 15mg/kg of subject body weight of anti-Ang-2 antibody may be administered after which one or more doses of VEGF antagonist may be administered to treat, alleviate, reduce or ameliorate one or more conditions associated with DME or AMD (*e.g.*, angiogenesis inhibition). In some embodiments, the anti-Ang-2 antibody is administered at one or more doses resulting in an improvement in one or more parameters (*e.g.*, retinal thickening, visual acuity) followed by the administration of a VEGF antagonist (*e.g.*, aflibercept) to prevent recurrence or have additive

activity. Alternative embodiments of the invention pertain to concomitant administration of anti-Ang-2 antibody and a VEGF antagonist which is administered at a separate dosage at a similar or different frequency relative to the anti-Ang-2 antibody. In some embodiments, the VEGF antagonist is administered before, after or concurrently with the anti-Ang-2 antibody. In certain embodiments, the VEGF antagonist is administered as a single dosage formulation with the anti-Ang-2 antibody.

Dosage

[00135] The amount of Ang-2 inhibitor (*e.g.*, anti- Ang-2 antibody) administered to a subject according to the methods of the present invention is, generally, a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" means an amount of Ang-2 inhibitor that results in one or more of: (a) a reduction in the severity of retinal vascular leak; (b) a reduction in the area of retinal or choroidal neo-vascularization; (c) change in central retinal thickness; (d) an increase in the duration of suppression of vascular leak in the eye; and (e) a reduction in the number of intravitreal injections in a subject having an eye disease or disorder associated with angiogenesis.

[00136] In the case of an anti- Ang-2 antibody, a therapeutically effective amount can be from about 0.05 mg to about 100 mg, *e.g.*, about 0.05 mg, about 0.1 mg, about 1.0 mg, about 1.5 mg, about 2.0 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, or about 100 mg, of the anti- Ang-2 antibody. In certain embodiments, 0.5 mg, 1.0 mg, 3.0 mg or 6.0 mg of an anti-Ang-2 antibody is administered.

[00137] The amount of Ang-2 inhibitor 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 Ang-2 inhibitor may be administered to a patient at a dose of about 0.0001 to about 100 mg/kg of patient body weight.

[00138] In certain embodiments, 0.5 mg, 1.0 mg, 3.0 mg or 6.0 mg of an anti-Ang-2 antibody is administered in combination with 0.05 mg to about 10 mg of a VEGF antagonist (*e.g.*, aflibercept).

EXAMPLES

[00139] 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.

Example 1: Effects of combined inhibition of VEGF and Ang2 using aflibercept (VEGF Trap) and anti-Ang2 antibody on the developing retinal angiogenesis in mice

[00140] Introduction: VEGF is the key modulator of angiogenesis in normal and pathological angiogenesis. However, other growth factors are also involved in angiogenesis and are able to mediate blood vessel resistance to anti-VEGF therapies. Angiopoietin-2 (Ang2) was shown to be involved in blood vessel growth and regression in various circumstances in a context-dependent manner. In this study we tested the effects of VEGF blockade using aflibercept alone and in combination with Ang2 inhibition with an anti-Ang2 antibody on blood vessel growth and regression in a normal retinal vascular development (RVD) model.

[00141] Human anti-Ang-2 antibodies were generated as described in US Patent Application Publication No. US20110027286. The exemplary anti-Ang-2 antibody used in the present and following Examples is the human anti-Ang-2 antibody designated as H1H685 with HCVR/LCVR of SEQ ID NOs: 1/2 (also referred to herein as "mAb1").

[00142] In a first experiment, anti-Ang-2 antibody alone or in combination with aflibercept was administered intravitreally.

[00143] Methods: Normal C57Bl/6 mouse pups were treated with aflibercept and anti-Ang2 antibody individually or in combination from postnatal day 4 (P4) to P6. Pups were injected IVT on P4 with 5µg of a control protein (hFc), 5µg mAb1 (anti-Ang2 antibody), or 1.25 µg aflibercept, as single agents, or mixture of both aflibercept and mAb1. Pups were humanely euthanized at P6, the eyes were removed and fixed in 4% paraformaldehyde. The retinas were then dissected and stained with FITC labeled GS Lectin I (for vascular endothelial cells).

[00144] Results: P4 was selected as the starting point for treatment with mAb1 to assess the effect of Ang2 and/or VEGF inhibition on RVD. Administration of mAb1 or aflibercept reduced the outgrowth of the superficial retinal vasculature compared to hFc treated controls. Specifically, mAb1 and aflibercept decreased the mean vascularized area of the retina by 17% and 36%, respectively, relative to hFc controls, while combined treatment with both mAb1 and aflibercept reduced vascular area by 72%, representing complete arrest of retinal vascular development over the treatment period (compared to retinal vascular area at P4). mAb1 and aflibercept also decreased the mean vessel length by 23% and 22%, respectively, compared to hFc controls. Combined treatment with mAb1 and aflibercept had a significant synergistic effect, leading to a dramatic 77% decrease in total vessel length. Total blood vessel length was even smaller by 25% in the combination treated samples compared to P4 retinas.

[00145] In a second experiment, doses of mAb1 (25 mg/kg, subcutaneous [SC]) and aflibercept (25 mg/kg, SC) utilized were in excess of the minimal doses required to obtain maximal

suppression of retinal angiogenesis when each drug is used as a single agent. Administration of either mAb1 or aflibercept on P3 significantly reduced the mean vascularized area of the retina measured at P6 by 36% and 42%, respectively, relative to hFc controls. Moreover, the mean vascularized area of the retina was significantly smaller in the animals treated with both mAb1 and aflibercept, compared to animals treated with either agent alone, being reduced by 68%, representing a near complete arrest of retinal vascular development over the treatment period.

[00146] Conclusions: Combined pharmacological inhibition of Ang2 and VEGF-A had a greater effect on developmental retinal angiogenesis than administration of Ang2 or VEGF-A blocker alone, such that inhibition of both Ang2 and VEGF-A resulted in a near completed arrest of retinal vascular development during the treatment period and partial blood vessel regression compared to the initial treatment conditions at P4.

Example 2: Effect of IVT injection of co-formulated mAb1 and aflibercept on DL-alpha-aminoadipic acid (DL-alpha-AAA) – induced retinal neovascularization (RNV) in rabbit eyes

[00147] The glial toxin DL-alpha-AAA targets retinal Muller cells and astrocytes and leads to neovascularization and chronic vascular leak lasting at least 12 months (Kato et al 1993; Neuroscience 57: 473). The purpose of this study was to evaluate the effects of IVT injection of co-formulated Anti-Ang2 and aflibercept on RNV induced by DL-alpha-AAA.

[00148] Methods: Male New Zealand White Rabbits (>2kg body weight) were treated with a single intravitreal injection of DL-alpha-AAA to induce RNV. The leak was monitored and quantitated non-invasively using fluorescein angiography (FA). Pathological vasculature and a relatively invariable leakage area were established over 13 weeks post injection. Upon disease establishment, the subjects were split into three groups for treatment, as shown below:

Group I: 125 µg/50 µl of aflibercept IVT

Group II: 500 µg/50 µl of mAb1 IVT

Group III: Co-formulated 125 µg aflibercept and 500 µg mAb1/50 µl, IVT

[00149] A baseline examination was performed prior to first treatment to balance treatment groups. Follow-up examinations were performed on wk 1, 2, 3, 4, 5, 6, 7, and 8 Post-IVT treatments. Leakage area was quantified using Adobe Photoshop after fluorescein angiography (FA) analysis. A fluorescent agent was administered intravenously to monitor vessel leak and ocular coherence tomography using light waves was used to monitor retinal structure.

[00150] Results: As shown in Figure 1, leakage was shown to return at week 3 in aflibercept-treated subjects, whereas leakage area did not change till week 8 in subjects treated with mAb1 and aflibercept combination. Co-treatment of aflibercept and mAb1 can significantly enhance the duration of anti-leak effects up to 3-fold compared to a single IVT injection of aflibercept alone.

Example 3: Systemic administration of mAb1 alone or co-treatment with VEGF Trap-Eye IVT on DL-AAA induced retinal neo-vascular leak in rabbit eyes

[00151] The purpose of this study was to evaluate the effects of co-treatment of Anti-Ang2 antibody and aflibercept on RNV induced by DL-alpha-AAA, wherein the intravitreal administration of aflibercept was followed by systemic administrations of mAb1. The rationale of this study was to try and maintain the suppression of leakage over longer periods of time with an initial IVT injection of aflibercept and follow up with systemic injections once in two weeks (q2w) of anti-Ang 2 antibody.

[00152] As disclosed in Example 2, retinal neovascularization was induced in male New Zealand rabbits with a single intravitreal injection of DL-alpha-AAA. Stable retinal neovascularization and vascular leak was established 10 weeks post induction. After ten weeks of disease establishment, the subjects were split into four groups with balanced leakage severity and treatment was administered as shown below:

Group 1: Control 42µg/50µl, IVT and Control 5 mg/kg, IV, q2w

Group 2: Control 42µg/50µl, IVT and mAb1 (anti-Ang2 ab) 15 mg/kg, IV, q2w

Group 3: Aflibercept 125µg/50µl, IVT and Control 5 mg/kg, IV, q2w

Group 4: Aflibercept 125µg/50µl, IVT and mAb1 (a-Ang2) 15 mg/kg, IV, q2w

[00153] Analysis of leakage area by Fluorescein Angiography (FA); Baseline fluorescein angiography (FA) and optic coherent tomography (OCT) prior to 1st treatment; Follow-up FA and OCT on Week 1, 2, 3, 4, 6, 8, 10, 12 and 14 Post-IV treatment

[00154] As shown in Figure 2, there was no leakage in aflibercept-treated eyes in week 2. Leakage was shown to return at week 4 with aflibercept treatment alone. However there was no leakage till week 10 in subjects treated with the combination of aflibercept and mAb1. Initial return of leakage was shown in week 12 in co-treated subjects.

Example 4: IVT Injection of aflibercept on DL-alpha-AAA induced RNV and vascular leak in rabbit eye

[00155] This study is a dose-ranging study on the effect of IVT aflibercept monotherapy on DL-alpha-AAA induced retinal neovascularization (RNV) and vascular leak in rabbit eye. As disclosed in Example 2, DL-alpha-AAA was used to induce RNV in rabbit eyes. Upon establishment of RNV and vascular leak, the subjects were treated with 4 doses of aflibercept: 50 mcg, 125 mcg, 250 mcg, and 500 mcg. Retinal vascular leak was monitored and quantitated by FA (as disclosed elsewhere herein) at follow-up time points on weeks 1, 2, 3, 4, 5, 6, 8 10 and 12 post-IVT.

[00156] Without treatment, FA showed that retinal NV and vascular leak remain consistent from weeks 10 to 22. Table 1 shows the mean time of leak recurrence after treatment wherein "first leak recurrence" = time for recurrence of any leak, not full recurrence.

Table 1: Mean time of first leak recurrence

IVT VTE dose	Mean (wks)	STD (wks)	Range (wks)
500 mcg (n=4)	9	2	8 – 12
250 mcg (n=3)	6.3	1.5	5 – 8
125 mcg (n=2)	4.5	0.7	4 – 5
50 mcg (n=2)	2.5	0.7	2 - 3

[00157] 500 mcg of aflibercept blocked retinal NV leak within 1 week, and leak did not resume until week 12 after treatment. Thus aflibercept treatment leads to suppression of vascular leak and partial regression of neovascularization. Vascular leakage reoccurs but length of suppression is dose dependent.

Example 5: Systemic administration of anti-Ang-2 antibody inhibits Matrigel-induced choroidal neovascularization (CNV) in rats

[00158] Introduction: Ang2 is a ligand for the tyrosine kinase receptor Tie-2 and is broadly expressed in the vascular endothelium of developing blood vessels and in vessels undergoing active growth or remodeling in diverse physiological and pathophysiological conditions. mAb1 is a human monoclonal, neutralizing antibody against Ang-2. The study utilized a rat model of choroidal neovascularization (CNV) to assess the inhibitory effects of mAb1-mediated pharmacological inhibition of Ang-2 on CNV.

[00159] **Methods:** CNV was induced in Sprague Dawley (SD) rats by a subretinal injection of Matrigel on Day 0. One group of animals (animals: N=4-6; eyes: N=8-12) was used to establish a baseline and animals were perfused with a dye [1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (DiI)] to stain vessels 10 days after CNV induction. The other animals were divided into two groups (animals: N=5-6, and eyes: N=10-12, in each group) and treated with masked solutions by subcutaneous injection of 25 mg/kg mAb1, or 6.5 mg/kg hFc at equimolar amount relative to mAb1, respectively, on days 10, 13, and 16 followed by perfusion with DiI to stain vessels on Day 20. Subretinal lesion and CNV vessel volumes were quantified from 50 µm sections throughout the entire lesion using the formula

$$V = T \times \sum_{i=1}^{n} A_i$$

wherein V : Total Lesion Volume;

T : Section Thickness;
A : Area of lesion or vessels on each section

[00160] The results from two identical experiments were combined for lesion volume, vessel volume, and vessel density comparison among three groups. A two-tailed Student t-test was used to compare the differences of lesion volume, vessel volume, and vessel density between groups treated with mAb1 versus control. Concentrations of functional mAb1 and levels of anti-mAb1 antibodies in rat serum were measured using a direct enzyme linked immunosorbent assay (ELISA).

[00161] Results: Systemic administration of mAb1 (25 mg/kg) to SD rats produced a suppression of the total lesion volume and vessel volume compared to hFc-treated control (Figure 3). In the control treated group, subretinal lesion volume, neovessel volume, and neovessel density increased by 28.2%, 62.5%, and 28.6%, respectively, by Day 20, relative to the baseline at Day 10. The mAb1 treated group showed 26.5% reduction of total lesion volume, and 26.9% reduction of vessel volume, compared to hFc treated group, though the vessel density was unchanged compared to the hFc treated group (Figure 3). Compared to hFc-treated controls, the mAb1 treated group showed statistically significant reduction of total lesion volume (Student t-test, p=0.0034), and a trend (26.9%) towards neovessel volume reduction, but this trend did not achieve statistical significance (Student t-test, p=0.1658, which may have been due to variation within the group. Bioanalytical analysis of functional mAb1 and anti-mAb1 antibodies in rat serum samples showed measurable concentrations of functional mAb1 in mAb1-treated animals with levels of 504 ±107 µg/mL on terminal date (Day 20) (Table 2). Individual anti-mAb1 responses were negative.

Table 2: Concentration of mAb1 and anti-mAb1 antibodies in rat serum samples

	mAb1 (µg/mL) (Mean±SD)	Anti-mAb1 antibody
IVT mAb1	504 ±107	(-)
IVT hFc	BLQ	(-)

BLQ: Below Limit of Quantification (<0.078 µg/mL)
(-): Antibody negative

[00162] Conclusions: The effects of mAb1, a neutralizing, human monoclonal antibody against Ang2, were evaluated in Matrigel induced CNV in SD rats. The results indicate that systemic (subcutaneous) treatment with mAb1 can significantly inhibit Matrigel induced CNV lesion in rats. Thus, this Example provides additional support for the use of Ang-2 inhibitors (such as mAb1) as a systemic monotherapy to treat vascular eye diseases.

Example 6: Formulation comprising an anti-Ang-2 antibody

[00163] Formulation development activities included the screening of buffers, organic co-solvents, and thermal stabilizers in liquid formulations of anti-Ang-2 antibody to identify excipients that enhance the stability of the protein. Buffer conditions were also examined to determine the optimal pH for maximum protein stability. Results generated from these studies were used to develop a stable, liquid formulation suitable for clinical use. The anti-Ang-2 antibody is the human anti-Ang-2 antibody with HCVR/LCVR of SEQ ID NOs: 1/2 and designated as H1H685 in US Patent Application Publication US20110027286 (also referred to herein as "mAb1").

[00164] The anti-Ang-2 antibody was formulated at four concentrations:

(i) 10mg/mL ± 1.5 mg/mL,

(ii) 20 mg/mL ± 3.0 mg/mL,

(iii) 60 mg/mL ± 9.0 mg/mL, and

(iv) 120 mg/mL ± 18.0 mg/mL.

[00165] In various embodiments, the anti-Ang-2 antibody is formulated in 10 ± 1.5 mM sodium phosphate (pH 6.2 ± 0.3), 0.03% ± 0.0045% polysorbate 20, 40 mM ± 6.0 mM sodium chloride, and 5% ± 0.75% sucrose, in water.

[00166] The stability of the formulated drug substance and drug product was assessed using the following assays:

[00167] Color and appearance by visual inspection; pH; turbidity measured by increase in optical density at 405nm; subvisible particulate analysis by microflow imaging (MFI); protein concentration by RP-HPLC; purity by size exclusion ultra-performance liquid chromatography (SE-UPLC); reduced and non-reduced SDS-PAGE; charged variant analysis by cation exchange UPLC; potency by bioassay. Additional details are described in Example 8.

[00168] Stability studies were initiated to determine the storage, accelerated (temperatures above storage conditions), and stress (agitation and freezing and thawing) stability of research lots of 10 mg/mL mAb1, and 120 mg/mL mAb1 FDS. These conditions were chosen to bracket the protein concentrations of FDS that used to manufacture the clinical DP. Evaluation of research lots of FDS under accelerated and stress conditions was performed by subjecting FDS to a variety of tests designed to exceed stresses the FDS may encounter during the manufacture of DP and to elucidate the degradation pathways for mAb1 FDS. FDS was filled in 5 mL polycarbonate vials

[00169] The research stability of the drug substance was studied as shown in Tables 3 and 4:

Table 3: Research Stability Study for mAb1 Formulated Drug Substance

Storage Stability[1)]		Container/Closure
Storage Temperature	**Length of Storage (months)**	5 mL Nalge-Nunc polycarbonate vial with lined closure
-80°C	0, 1, 3, 6, 9, 12, 18, 24, and 36	
-30°C	0, 1, 3, 6, 9, 12, 18, 24, and 36	

Storage Stability[1)]		Container/Closure
-20°C	0, 1, 3, 6, 9, 12, 18, 24, and 36	
Accelerated Stability[2)]		
Incubation Condition	**Length of Incubation**	
5°C	0, 14, 28, and 56 days	
25°C/60% RH	0, 7, 14, and 28 days	
40°C/75% RH	0, 7, 14, and 28 days	
Stress Stability		
Stress	**Duration of Stress**	
Agitation (vortex)	0, 60, and 120 minutes	
Freeze/Thaw[3)]	0, 4, and 8 cycles	

Table 4: Analysis Plan for mAb1 Formulated Drug Substance Research Stability Study

Assay	Samples to be Analyzed
Color and Appearance	All Samples
pH	All Samples
Turbidity (Increase in OD at 405 nm)	All Samples
% mAb1 Recovered by RP-HPLC	All Samples
% mAb1 Purity by Non-Reduced and Reduced SDS-PAGE	t = 0, 6, 12, 24 and 36 months at -80°C, -30°C, and -20°C 56 days at 5°C, 28 days at 25°C/60% RH and 40°C/75% RH 120 min Agitation, 8X Freeze/Thaw
% Purity by SE-UPLC	All Samples
Charge Variant Analysis by CEX-UPLC	All Samples
Charge Variant Analysis by iCIEF	All Samples
% mAb1 Relative Potency by Bioassay	t = 0, 6, 12, 24 and 36 months at -80°C and -20°C 56 days at 5°C, 28 days at 25°C/60% RH and 40°C/75% RH 120 min Agitation, 8X Freeze/Thaw

[00170] The results of the stability studies are summarized in Tables 5 – 14 below:

Table 5: Research Stability of 10 mg/mL mAb1 Formulated Drug Substance Stored at -80°C

Formulation	**10 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume	**1.0 mL**					
Container/Closure	**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
	Length of Storage (months)					
Assay	**0**	**1**	**3**	**6**	**9**	**12**
Color and Appearance	Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)	0.00	0.00	0.00	0.00	0.00	0.00

Formulation		**10 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
		Length of Storage (months)					
Assay		**0**	**1**	**3**	**6**	**9**	**12**
pH		6.2	6.2	6.2	6.3	6.2	6.2
% Total mAb1 Recovered by RP-HPLC		100	100	104	104	104	100
Purity by SDS-PAGE	Non-reduced; % main peak	95	NR	NR	94	NR	95
	Reduced; % heavy + light chain	100	NR	NR	100	NR	100
Purity by SE-UPLC	% HMW	2.3	2.4	2.4	2.4	2.4	2.4
	% Native	96.8	96.4	96.5	96.4	96.6	96.7
	% LMW	0.9	1.2	1.1	1.2	1.0	0.9
Charge Variant Analysis by CEX-UPLC	% Acidic	35.9	35.3	35.6	35.1	33.4	34.5
	% Main	56.2	57.0	56.7	57.8	58.0	58.1
	% Basic	7.8	7.7	7.8	7.2	8.6	7.4
Charge Variant Analysis by iCIEF	% Acidic	33.8	33.1	32.7	34.4	34.5	33.2
	% Main	59.9	59.9	60.7	59.7	59.6	59.9
	% Basic	6.3	7.0	6.6	5.9	5.9	7.0
% mAb1Relative Potency by Bioassay		95	NR	NR	144	NR	99

[00171] Table 6: Research Stability of 10 mg/mL mAb1 Formulated Drug Substance Stored at -30℃

Formulation	**10 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume	**1.0 mL**					
Container/Closure	**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
	Length of Storage (months)					
Assay	**0**	**1**	**3**	**6**	**9**	**12**
Color and Appearance	Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)	0.00	0.00	0.00	0.00	0.00	0.00
pH	6.2	6.1	6.2	6.2	6.2	6.2

Formulation		**10 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
		Length of Storage (months)					
Assay		**0**	**1**	**3**	**6**	**9**	**12**
% Total mAb1 Recovered by RP-HPLC		100	100	103	104	104	100
Purity by SDS-PAGE	Non-reduced; % main peak	95	NR	NR	96	NR	95
	Reduced; % heavy + light chain	100	NR	NR	100	NR	100
Purity by SE-UPLC	% HMW	2.3	2.4	2.3	2.4	2.3	2.3
	% Native	96.8	96.5	96.8	96.4	96.8	96.8
	% LMW	0.9	1.1	0.9	1.2	1.0	0.9
Charge Variant Analysis by CEX-UPLC	% Acidic	35.9	35.5	35.4	35.1	33.6	34.8
	% Main	56.2	56.8	56.9	57.9	58.5	58.0
	% Basic	7.8	7.8	7.7	7.1	8.0	7.2
Charge Variant Analysis by iCIEF	% Acidic	33.8	34.0	32.9	34.1	34.3	33.2
	% Main	59.9	60.0	61.2	60.2	59.1	60.3
	% Basic	6.3	6.0	5.9	5.7	6.5	6.5
% mAb1 Relative Potency by Bioassay		95	NR	NR	NR	NR	NR

[00172] Table 7: Research Stability of 10 mg/mL mAb1 Formulated Drug Substance Stored at -20℃

Formulation	**10 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume	**1.0 mL**					
Container/Closure	**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
	Length of Storage (months)					
Assay	**0**	**1**	**3**	**6**	**9**	**12**
Color and Appearance	Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)	0.00	0.00	0.00	0.00	0.00	0.00
pH	6.2	6.2	6.2	6.2	6.3	6.2
% Total **mAb1** Recovered by RP-HPLC	100	104	103	106	105	100

Formulation		**10 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
		Length of Storage (months)					
Assay		**0**	**1**	**3**	**6**	**9**	**12**
Purity by SDS-PAGE	Non-reduced; % main peak	95	NR	NR	96	NR	95
	Reduced; % heavy + light chain	100	NR	NR	100	NR	99
Purity by SE-UPLC	% HMW	2.3	2.1	2.0	1.9	1.8	1.9
	% Native	96.8	96.7	96.8	96.8	97.3	97.3
	% LMW	0.9	1.2	1.2	1.2	1.0	0.9
Charge Variant Analysis by CEX-UPLC	% Acidic	35.9	35.4	35.5	34.9	33.5	34.3
	% Main	56.2	56.8	56.9	57.9	58.7	58.1
	% Basic	7.8	7.7	7.6	7.3	7.9	7.6
Charge Variant Analysis by iCIEF	% Acidic	33.8	32.7	34.1	34.2	34.1	34.4
	% Main	59.9	60.6	59.5	60.0	60.1	59.5
	% Basic	6.3	6.4	6.3	5.8	5.7	6.2
% **mAb1** Relative Potency by Bioassay		95	NR	NR	94	NR	85

[00173] Table 8: Research Stability of 120 mg/mL mAb1 Formulated Drug Substance Stored at -80℃

Formulation		**120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
		Length of Storage (months)					
Assay		**0**	**1**	**3**	**6**	**9**	**12**
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00	0.00
pH		6.2	6.3	6.3	6.3	6.3	6.2
% Total **mAb1** Recovered by RP-HPLC		100	99	101	105	101	103
Purity by SDS-PAGE	Non-reduced; % main peak	96	NR	NR	96	NR	96

Formulation		**120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
Assay		**Length of Storage (months)**					
		0	**1**	**3**	**6**	**9**	**12**
	Reduced; % heavy + light chain	100	NR	NR	100	NR	99
Purity by SE-UPLC	% HMW	2.6	2.7	2.6	2.7	2.6	2.7
	% Native	96.4	96.2	96.4	96.2	96.5	96.5
	% LMW	1.0	1.1	1.0	1.2	0.9	0.8
Charge Variant Analysis by CEX-UPLC	% Acidic	35.7	35.3	35.2	35.0	33.3	34.6
	% Main	55.8	56.5	56.5	57.4	58.2	57.8
	% Basic	8.5	8.2	8.3	7.7	8.6	7.6
Charge Variant Analysis by iCIEF	% Acidic	33.6	33.9	32.8	34.2	32.7	33.9
	% Main	59.9	59.9	60.8	59.2	60.7	59.8
	% Basic	6.5	6.2	6.5	6.6	6.6	6.4
% mAb1 Relative Potency by Bioassay		118	NR	NR	74	NR	89

[00174] Table 9: Research Stability of 120 mg/mL mAb1 Formulated Drug Substance Stored at -30℃

Formulation		**120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
Assay		**Length of Storage (months)**					
		0	**1**	**3**	**6**	**9**	**12**
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00	0.00
pH		6.2	6.3	6.3	6.3	6.3	6.3
% Total **mAb1** Recovered by RP-HPLC		100	98	103	105	100	104
Purity by SDS-PAGE	Non-reduced; % main peak	96	NR	NR	96	NR	97
	Reduced; % heavy + light chain	100	NR	NR	100	NR	100

Formulation		**120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
		Length of Storage (months)					
Assay		**0**	**1**	**3**	**6**	**9**	**12**
Purity by SE-UPLC	% HMW	2.6	2.6	2.6	2.6	2.6	2.6
	% Native	96.4	96.2	96.3	96.2	96.6	96.6
	% LMW	1.0	1.2	1.1	1.1	0.9	0.8
Charge Variant Analysis by CEX-UPLC	% Acidic	35.7	35.5	35.2	34.8	33.4	34.3
	% Main	55.8	56.3	56.0	56.9	58.1	57.6
	% Basic	8.5	8.2	8.8	8.3	8.5	8.2
Charge Variant Analysis by iCIEF	% Acidic	33.6	32.9	33.4	33.4	34.1	32.4
	% Main	59.9	60.4	60.1	60.8	59.6	61.1
	% Basic	6.5	6.7	6.5	5.7	6.3	6.5
% mAb1 Relative Potency by Bioassay		118	NR	NR	NR	NR	NR

[00175] Table 10: Research Stability of 120 mg/mL mAb1 Formulated Drug Substance Stored at -20℃

Formulation		**120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
		Length of Storage (months)					
Assay		**0**	**1**	**3**	**6**	**9**	**12**
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00	0.00
pH		6.2	6.3	6.3	6.3	6.3	6.3
% Total **mAb1** Recovered by RP-HPLC		100	98	100	105	103	105
Purity by SDS-PAGE	Non-reduced; % main peak	96	NR	NR	96	NR	96
	Reduced; % heavy + light chain	100	NR	NR	100	NR	100
Purity by SE-UPLC	% HMW	2.6	2.6	2.5	2.7	2.6	2.7
	% Native	96.4	96.3	96.9	96.2	96.5	96.5

Formulation		**120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
Assay		**Length of Storage (months)**					
		0	**1**	**3**	**6**	**9**	**12**
	% LMW	1.0	1.1	0.7	1.1	0.9	0.8
Charge Variant Analysis by CEX-UPLC	% Acidic	35.7	35.4	35.2	34.7	33.1	34.2
	% Main	55.8	56.2	56.5	57.2	58.2	57.5
	% Basic	8.5	8.4	8.4	8.1	8.7	8.3
Charge Variant Analysis by iCIEF	% Acidic	33.6	33.5	32.6	33.2	33.7	33.6
	% Main	59.9	60.7	61.8	60.6	60.2	59.8
	% Basic	6.5	5.8	5.6	6.2	6.1	6.5
% **mAb1** Relative Potency by Bioassay		118	NR	NR	80	NR	102

[00176] Table 11: Research Stability of 10 mg/mL mAb1 Formulated Drug Substance –Effect of Accelerated Conditions

Formulation		**10 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**									
Fill Volume		**1.0 mL**									
Container/Closure		**5 mL polycarbonate vial w/ silicone lined polypropylene screw cap**									
		Storage Condition/Length of Storage (days)									
		No Storage	**5°C**			**25°C/60%RH**			**40°C/75%RH**		
Assay		**0**	**14**	**28**	**56**	**7**	**14**	**28**	**7**	**14**	**28**
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pH		6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.1
% Total **mAb1** Recovered by RP-HPLC		100	105	103	105	104	106	109	104	109	121[1)]
Purity by SDS-PAGE	Non-reduced; % main peak	95	NR	NR	95	NR	NR	91	NR	NR	84
	Reduced; % heavy + light chain	100	NR	NR	100	NR	NR	100	NR	NR	99
Purity by SE-UPLC	% HMW	2.3	1.9	1.8	1.8	1.7	1.6	1.7	1.7	1.7	2.2
	% Native	96.8	97.2	97.2	97.3	97.3	97.3	97.1	97.1	96.8	95.6
	% LMW	0.9	0.9	1.0	0.9	1.0	1.0	1.2	1.2	1.5	2.2
Charge	% Acidic	35.9	35.9	35.9	35.4	36.1	37.0	38.6	41.0	46.9	56.0

Formulation		**10 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**									
Fill Volume		**1.0 mL**									
Container/Closure		**5 mL polycarbonate vial w/ silicone lined polypropylene screw cap**									
		Storage Condition/Length of Storage (days)									
		No Storage	**5°C**			**25°C/60%RH**			**40°C/75%RH**		
Assay		**0**	**14**	**28**	**56**	**7**	**14**	**28**	**7**	**14**	**28**
Variant Analysis by CEX-UPLC	% Main	56.2	56.5	56.9	57.1	55.9	55.4	54.0	50.8	46.1	37.4
	% Basic	7.8	7.7	7.2	7.5	8.0	7.6	7.5	8.2	7.0	6.6
Charge Variant Analysis by iCIEF	% Acidic	33.8	33.9	33.0	33.9	33.7	35.3	37.0	40.1	45.7	55.1
	% Main	59.9	59.6	60.7	59.5	59.6	57.8	55.6	52.5	46.3	37.2
	% Basic	6.3	6.4	6.3	6.6	6.7	6.9	7.3	7.4	8.1	7.7
% **mAb1** Relative Potency by Bioassay		95	NR	NR	101	NR	NR	90	NR	NR	136

[00177] Table 12: Research Stability of 120 mg/mL mAb1 Formulated Drug Substance – Effect of Accelerated Conditions

Formulation		**120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**									
Fill Volume		**1.0 mL**									
Container/Closure		**5 mL polycarbonate vial w/ silicone lined polypropylene screw cap**									
		Storage Condition/Length of Storage (days)									
		No Storage	**5°C**			**25°C/60%RH**			**40°C/75%RH**		
Assay		**0**	**14**	**28**	**56**	**7**	**14**	**28**	**7**	**14**	**28**
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pH		6.2	6.3	6.3	6.2	6.3	6.3	6.2	6.3	6.3	6.2
% Total **mAb1** Recovered by RP-HPLC		100	102	101	102	102	104	104	104	105	107
Purity by SDS-PAGE	Non-reduced; % main peak	96	NR	NR	96	NR	NR	92	NR	NR	88
	Reduced; % heavy + light chain	100	NR	NR	100	NR	NR	100	NR	NR	100
Purity by SE-UPLC	% HMW	2.6	2.6	2.5	2.7	2.7	2.9	3.0	3.8	4.9	6.5
	% Native	96.4	96.5	96.5	96.4	96.5	96.3	95.9	95.0	93.5	91.5
	% LMW	1.0	0.9	0.9	0.9	0.8	0.8	1.1	1.2	1.7	1.9
Charge Variant Analysis by CEX-UPLC	% Acidic	35.7	35.3	35.6	34.8	35.9	36.1	37.5	39.7	44.9	53.3
	% Main	55.8	56.2	56.2	56.6	55.4	55.0	53.0	50.6	46.2	37.8
	% Basic	8.5	8.5	8.2	8.5	8.7	8.9	9.5	9.7	9.0	8.9

Formulation		**120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**									
Fill Volume		**1.0 mL**									
Container/Closure		**5 mL polycarbonate vial w/ silicone lined polypropylene screw cap**									
		Storage Condition/Length of Storage (days)									
		No Storage	**5°C**			**25°C/60%RH**			**40°C/75%RH**		
Assay		**0**	**14**	**28**	**56**	**7**	**14**	**28**	**7**	**14**	**28**
Charge Variant Analysis by iCIEF	% Acidic	33.6	34.4	32.3	34.2	33.2	34.2	36.1	38.4	44.8	54.2
	% Main	59.9	60.0	61.5	59.5	60.3	58.9	56.7	53.9	46.6	37.4
	% Basic	6.5	5.5	6.1	6.3	6.5	6.9	7.2	7.8	8.6	8.4
% **mAb1** Relative Potency by Bioassay		118	NR	NR	94	NR	NR	96	NR	NR	123

[00178] Table 13: Research Stability of 10 mg/mL mAb1 Formulated Drug Substance –Effect of Stress Conditions

Formulation		**10 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**				
Fill Volume		**1.0 mL**				
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**				
		Stress Condition/Length of Stress				
		No Stress	**Agitation (minutes)**		**Freeze/Thaw (Cycles)**	
Assay		**0**	**60**	**120**	**4**	**8**
Color and Appearance		Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00
pH		6.2	6.3	6.3	6.2	6.2
% Total **mAb1** Recovered by RP-HPLC		100	96	97	103	105
Purity by SDS-PAGE	Non-reduced; % main peak	95	NR	95	NR	95
	Reduced; % heavy + light chain	100	NR	100	NR	100
Purity by SE-UPLC	% HMW	2.3	2.0	2.0	2.4	2.3
	% Native	96.8	97.1	97.3	96.7	96.7
	% LMW	0.9	0.9	0.8	0.9	0.9
Charge Variant Analysis by CEX-UPLC	% Acidic	35.9	35.7	35.6	35.5	35.6
	% Main	56.2	56.2	55.9	56.2	56.3
	% Basic	7.8	8.1	8.5	8.4	8.1
Charge Variant	% Acidic	33.8	34.0	33.9	34.1	34.2

Formulation		10 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2				
Fill Volume		**1.0 mL**				
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**				
		Stress Condition/Length of Stress				
		No Stress	**Agitation (minutes)**		**Freeze/Thaw (Cycles)**	
Assay		**0**	**60**	**120**	**4**	**8**
Analysis by iCIEF	% Main	59.9	59.5	60.4	59.8	59.6
	% Basic	6.3	6.5	5.7	6.1	6.3
% **mAb1** Relative Potency by Bioassay		95	NR	103	NR	107

[00179] Table 14: Research Stability of 120 mg/mL mAb1 Formulated Drug Substance – Effect of Stress Conditions

Formulation		120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2				
Fill Volume		**1.0 mL**				
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**				
		Stress Condition/Length of Stress				
		No Stress	**Agitation (minutes)**		**Freeze/Thaw (Cycles)**	
Assay		**0**	**60**	**120**	**4**	**8**
Color and Appearance		Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00
pH		6.2	6.3	6.3	6.3	6.3
% Total **mAb1** Recovered by RP-HPLC		100	94	95	100	100
Purity by SDS-PAGE	Non-reduced; % main peak	96	NR	96	NR	95
	Reduced; % heavy + light chain	100	NR	100	NR	100
Purity by SE-UPLC	% HMW	2.6	2.7	2.8	2.8	3.0
	% Native	96.4	96.4	96.4	96.3	96.2
	% LMW	1.0	0.9	0.9	0.9	0.8
Charge Variant Analysis by CEX-UPLC	% Acidic	35.7	35.8	35.8	35.8	35.7
	% Main	55.8	55.9	56.2	56.1	55.8
	% Basic	8.5	8.4	8.0	8.1	8.4
Charge Variant Analysis by iCIEF	% Acidic	33.6	33.2	33.2	33.9	33.7
	% Main	59.9	60.4	60.3	59.5	60.1
	% Basic	6.5	6.4	6.5	6.6	6.2

Formulation	**120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**				
Fill Volume	**1.0 mL**				
Container/Closure	**5 mL polycarbonate vial with silicone lined polypropylene screw cap**				
	Stress Condition/Length of Stress				
	No Stress	**Agitation (minutes)**		**Freeze/Thaw (Cycles)**	
Assay	**0**	**60**	**120**	**4**	**8**
% **mAb1** Relative Potency by Bioassay	118	NR	120	NR	98

[00180] Research stability studies for the mAb1 drug products were conducted as shown in Tables 15 and 16:

Table 15: Research Stability Studies for mAb1 Drug Products

Storage Stability[1)]		**Container/Closure**
Storage Temperature	**Length of Storage (months)**	Type 1 borosilicate glass with FluroTec® coated 4432/50 butyl rubber stopper
5°C	0, 1, 3, 6, 9, 12, 18, 24, and 36	
Accelerated Stability[2)]		
Incubation Condition	**Length of Incubation**	
25°C	0, 1, 3, and 6 months	
37°C	0, 7, 14, and 28 days	
Stress Stability		
Stress	**Duration of Stress**	
Agitation (vortex)	0, 60, and 120 minutes	
Freeze/Thaw[3)]	0, 4, and 8 cycles	

Table 16: Research Stability Study Analysis Plan for mAb1 Drug Products

Assay	**Samples to be Analyzed**
Color and Appearance	All Samples
pH	All Samples
Turbidity (Increase in OD at 405 nm)	All Samples
% **mAb1** Recovered by RP-HPLC	All Samples
% **mAb1** Purity by Non-Reduced and Reduced SDS-PAGE	t = 0, 6, 12, 24 and 36 months at 5°C; 6 months at 25°C; 28 days at 37°C 120 min Agitation, 8X Freeze/Thaw
% Purity by SE-UPLC	All Samples
Charge Variant Analysis by CEX-UPLC	All Samples
Charge Variant Analysis by iCIEF	All Samples
Particulate Matter Analysis by MFI	t = 0, 6, 12, 24 and 36 months at 5°C; 6 months at 25°C; 28 days at 37°C 120 min Agitation, 8X Freeze/Thaw
% **mAb1** Relative Potency by Bioassay	t = 0, 6, 12, 24 and 36 months at 5°C;

Assay	Samples to be Analyzed
	6 months at 25°C; 28 days at 37°C 120 min Agitation, 8X Freeze/Thaw

[00181] The results of stability studies for the drug products are summarized in Tables 17 – 22:

Table 17: Research Stability of 10 mg/mL mAb1 Drug Product Stored at 5°C

Formulation		**10 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**				
Fill Volume		**0.5 mL**				
Container/Closure		**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**				
		Length of Storage (months)				
Assay		**0**	**1**	**3**	**6**	**9**
Color and Appearance		Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00
pH		6.3	6.3	6.2	6.2	6.3
% **mAb1** Recovered by RP-HPLC		100	102	99	97	98
Purity by SDS-PAGE	Non-reduced; % main peak	95	NR	NR	94	NR
	Reduced; % heavy + light chain	100	NR	NR	100	NR
Purity by SE-UPLC	% HMW	1.4	1.4	1.3	1.3	1.2
	% Native	97.7	97.6	97.6	97.5	97.8
	% LMW	1.0	1.1	1.1	1.1	1.0
Charge Variant Analysis by CEX-UPLC	% Acidic	39.3	39.1	37.5	37.3	38.5
	% Main	54.6	55.2	55.9	56.4	55.9
	% Basic	6.2	5.7	6.7	6.3	5.7
Charge Variant Analysis by iCIEF	% Acidic	37.6	38.3	37.5	38.4	37.3
	% Main	57.7	57.0	58.3	57.4	58.0
	% Basic	4.7	4.7	4.2	4.2	4.7
Particulate Analysis by MFI (particles/mL)	≥ 2 µm	6790	NR	NR	6905	NR
	≥ 10 µm	255	NR	NR	266	NR
	≥ 25 µm	44	NR	NR	26	NR
% **mAb1** Relative Potency by Bioassay		94	NR	NR	114	NR

[00182] **Table 18: Research Stability of 120 mg/mL mAb1 Drug Product Stored at 5°C**

Formulation		**120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**				
Fill Volume		**0.5 mL**				
Container/Closure		**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**				
		Length of Storage (months)				
Assay		**0**	**1**	**3**	**6**	**9**
Color and Appearance		Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00
pH		6.3	6.3	6.3	6.3	6.3
% **mAb1** Recovered by RP-HPLC		100	98	97	101	99
Purity by SDS-PAGE	Non-reduced; % main peak	95	NR	NR	94	NR
	Reduced; % heavy + light chain	100	NR	NR	100	NR
Purity by SE-UPLC	% HMW	2.6	2.7	2.8	3.1	3.0
	% Native	96.5	96.5	96.3	96.0	96.2
	% LMW	0.9	0.8	0.9	0.9	0.9
Charge Variant Analysis by CEX-UPLC	% Acidic	38.8	38.7	36.7	36.4	38.3
	% Main	54.3	54.3	55.9	55.4	56.0
	% Basic	6.9	7.0	7.4	8.2	5.7
Charge Variant Analysis by iCIEF	% Acidic	37.7	37.7	38.0	37.7	37.7
	% Main	58.0	57.9	57.5	57.6	57.6
	% Basic	4.3	4.4	4.5	4.7	4.7
Particulate Analysis by MFI (particles/mL)	≥ 2 µm	1995	NR	NR	12705	NR
	≥ 10 µm	111	NR	NR	282	NR
	≥ 25 µm	13	NR	NR	9	NR
% **mAb1** Relative Potency by Bioassay		107	NR	NR	150	NR

[00183] Table 19: Research Stability of 10 mg/mL mAb1 Drug Product – Effect of Accelerated Conditions

Formulation	**10 mg/mL** mAb1**, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**
Fill Volume	**0.5 mL**
Container/Closure	**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**

Assay		Storage Condition/Length of Storage						
		No Storage	25°C (months)			37°C (days)		
		0	1	3	6	7	14	28
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00	0.00	0.00
pH		6.3	6.2	6.2	6.2	6.3	6.3	6.3
% **mAb1** Recovered by RP-HPLC		100	105	99	98	100	105	98
Purity by SDS-PAGE	Non-reduced; % main peak	95	NR	NR	89	NR	NR	86
	Reduced; % heavy + light chain	100	NR	NR	100	NR	NR	96
Purity by SE-UPLC	% HMW	1.4	1.3	1.4	1.3	1.2	1.3	1.3
	% Native	97.7	97.4	96.8	96.5	97.1	97.0	96.2
	% LMW	1.0	1.3	1.8	2.2	1.7	1.7	2.6
Charge Variant Analysis by CEX-UPLC	% Acidic	39.3	41.4	48.1	53.9	41.9	45.3	52.1
	% Main	54.6	52.5	46.1	40.6	52.0	48.6	42.1
	% Basic	6.2	6.1	5.8	5.5	6.1	6.1	5.8
Charge Variant Analysis by iCIEF	% Acidic	37.6	40.7	47.3	54.7	40.1	44.2	51.7
	% Main	57.7	53.4	47.5	39.2	54.7	50.2	41.8
	% Basic	4.7	5.8	5.2	6.1	5.2	5.6	6.5
Particulate Analysis by MFI (particles/mL)	≥ 2 µm	6790	NR	NR	8385	NR	NR	3697
	≥ 10 µm	255	NR	NR	280	NR	NR	153
	≥ 25 µm	44	NR	NR	23	NR	NR	21
% Relative Potency by Bioassay		94	NR	NR	149	NR	NR	95

[00184] Table 20: Research Stability of 120 mg/mL mAb1 Drug Product – Effect of Accelerated Conditions

Formulation		**120 mg/mL** mAb1, **10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**						
Fill Volume		**0.5 mL**						
Container/Closure		**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**						
Assay		**Storage Condition/Length of Storage**						
		No Storage	**25°C (months)**			**37°C (days)**		
		0	**1**	**3**	**6**	**7**	**14**	**28**
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405		0.00	0.00	0.00	0.01	0.00	0.00	0.00
pH		6.3	6.3	6.3	6.3	6.3	6.3	6.3
% **mAb1** Recovered by RP-HPLC		100	98	96	100	96	104	95
Purity by SDS-PAGE	Non-reduced; % main peak	95	NR	NR	88	NR	NR	89

Formulation		**120 mg/mL** mAb1, **10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**						
Fill Volume		**0.5 mL**						
Container/Closure		**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**						
Assay		**Storage Condition/Length of Storage**						
		No Storage	**25°C (months)**			**37°C (days)**		
		0	**1**	**3**	**6**	**7**	**14**	**28**
	Reduced; % heavy + light chain	100	NR	NR	100	NR	NR	100
Purity by SE-UPLC	% HMW	2.6	3.0	3.2	4.2	3.3	3.5	3.8
	% Native	96.5	95.2	95.2	93.6	95.1	95.2	94.1
	% LMW	0.9	1.9	1.6	2.2	1.7	1.3	2.1
Charge Variant Analysis by CEX-UPLC	% Acidic	38.8	40.5	44.3	51.8	40.7	44.0	50.6
	% Main	54.3	51.9	47.1	40.8	51.2	48.0	41.5
	% Basic	6.9	7.6	8.6	7.4	8.1	8.0	7.9
Charge Variant Analysis by iCIEF	% Acidic	37.7	39.9	46.9	54.4	40.5	44.6	50.8
	% Main	58.0	55.0	47.1	39.4	54.6	49.2	42.5
	% Basic	4.3	5.1	6.0	6.2	4.9	6.2	6.7
Particulate Analysis by MFI (particles/mL)	≥ 2 µm	1995	NR	NR	7414	NR	NR	7027
	≥ 10 µm	111	NR	NR	179	NR	NR	193
	≥ 25 µm	13	NR	NR	13	NR	NR	5
% Relative Potency by Bioassay		107	NR	NR	120	NR	NR	145

[00185] Table 21: Research Stability of mAb1 (10 mg/mL) Drug Product – Effect of Stress Conditions

Formulation		**10 mg/mL** mAb1, **10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**				
Fill Volume		**0.5 mL**				
Container/Closure		**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**				
Assay		**Stress Condition/Length of Stress**				
		No Stress	**Agitation (minutes)**		**Freeze/Thaw (Cycles)**	
		0	**60**	**120**	**4**	**8**
Color and Appearance		Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00
pH		6.3	6.3	6.3	6.3	6.3
% **mAb1** Recovered by RP-HPLC		100	103	103	98	99
Purity by SDS-PAGE	Non-reduced; % main peak	95	NR	95	NR	95

Formulation		**10 mg/mL** mAb1**, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**				
Fill Volume		**0.5 mL**				
Container/Closure		**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**				
Assay		**Stress Condition/Length of Stress**				
		No Stress	**Agitation (minutes)**		**Freeze/Thaw (Cycles)**	
		0	**60**	**120**	**4**	**8**
	Reduced; % heavy + light chain	100	NR	100	NR	100
Purity by SE-UPLC	% Total HMW	1.4	1.5	1.4	1.5	1.6
	% Total Native	97.7	97.5	97.6	97.7	97.4
	% Total LMW	1.0	1.0	1.1	0.8	1.1
Charge Variant Analysis by CEX-UPLC	% Acidic	39.3	39.1	39.1	39.2	39.3
	% Main	54.6	54.7	54.6	54.9	54.5
	% Basic	6.2	6.2	6.2	5.9	6.2
Charge Variant Analysis by iCIEF	% Acidic	37.6	37.3	37.1	38.2	38.6
	% Main	57.7	58.8	58.9	57.2	56.4
	% Basic	4.7	3.8	4.0	4.6	5.0
Particulate Analysis by MFI (particles/mL)	≥ 2 µm	6790	NR	6089	NR	16535
	≥ 10 µm	255	NR	284	NR	236
	≥ 25 µm	44	NR	36	NR	42
% Relative Potency by Bioassay		94	NR	110	NR	73

[00186] Table 22: Research Stability of mAb1 (120 mg/mL) Drug Product – Effect of Stress Conditions

Formulation	**120 mg/mL** mAb1**, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**				
Fill Volume	**0.5 mL**				
Container/Closure	**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**				
	Stress Condition/Length of Stress				
	No Stress	**Agitation (minutes)**		**Freeze/Thaw (Cycles)**	
Assay	**0**	**60**	**120**	**4**	**8**
Color and Appearance	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)	0.00	0.00	0.00	0.00	0.00
pH	6.3	6.3	6.3	6.3	6.3
% **mAb1** Recovered by RP-HPLC	100	99	99	96	96

Formulation		**120 mg/mL mAb1, 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, 0.03% (w/v) polysorbate 20, pH 6.2**				
Fill Volume		**0.5 mL**				
Container/Closure		**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**				
		Stress Condition/Length of Stress				
		No Stress	**Agitation (minutes)**		**Freeze/Thaw (Cycles)**	
Assay		**0**	**60**	**120**	**4**	**8**
Purity by SDS-PAGE	Non-reduced; % main peak	95	NR	94	NR	93
	Reduced; % heavy + light chain	100	NR	100	NR	100
Purity by SE-UPLC	% Total HMW	2.6	2.6	2.6	3.0	3.0
	% Total Native	96.5	96.3	96.3	96.2	96.2
	% Total LMW	0.9	1.2	1.2	0.8	0.8
Charge Variant Analysis by CEX-UPLC	% Acidic	38.8	39.0	38.8	39.2	39.1
	% Main	54.3	54.5	54.6	53.6	53.8
	% Basic	6.9	6.5	6.6	7.3	7.2
Charge Variant Analysis by iCIEF	% Acidic	37.7	37.4	37.2	36.8	37.8
	% Main	58.0	58.5	58.2	58.5	57.4
	% Basic	4.3	4.1	4.5	4.7	4.8
Particulate Analysis by MFI (particles/mL)	≥ 2 µm	108	NR	891	NR	6365
	≥ 10 µm	11	NR	93	NR	175
	≥ 25 µm	0	NR	30	NR	19
% Relative Potency by Bioassay		107	NR	125	NR	111

[00187] The results of the stability studies indicated that:

[00188] Formulated drug substance (FDS) 10 mg/mL mAb1 is stable when stored at ≤ -20^{0}C for at least 12 months

[00189] FDS 120mg/mL mAb1 is stable when stored at ≤ -20^{0}C for at least 12 months

[00190] FDS 10 mg/mL mAb1 was physically and chemically stable after 56 days of incubation at 5°C and maintained potency when incubated at 5°C for 56 days or at 25°C/60% relative humidity (RH) and at 40°C/75% RH for 28 days.

[00191] FDS 120 mg/mL mAb1 was physically and chemically stable after 56 days of incubation at 5°C and maintained potency when incubated at 5°C for 56 days or at 25°C/60% relative humidity (RH) and at 40°C/75% RH for 28 days.

[00192] 10 mg/mL mAb1 FDS was physically and chemically stable when agitated (vortexed at ambient temperature) for 120 minutes or when subjected to 8 freeze/thaw cycles (freezing at -30°C and thawing at room temperature). No appreciable change in the physical or chemical stability was detected in any of the monitored attributes. mAb1 maintained potency when the 10 mg/mL mAb1 FDS was agitated (vortexed at ambient temperature) for 120 minutes or when subjected to 8 freeze/thaw cycles (freezing at -30°C and thawing at room temperature).

[00193] 120 mg/mL mAb1 FDS was physically and chemically stable when agitated (vortexed at ambient temperature) for 120 minutes or when subjected to 8 freeze/thaw cycles (freezing at -30°C and thawing at room temperature). No appreciable change in the physical or chemical stability was detected in any of the monitored attributes. mAb1 maintained potency when the 120 mg/mL mAb1 FDS was agitated (vortexed at ambient temperature) for 120 minutes or when subjected to 8 freeze/thaw cycles (freezing at -30°C and thawing at room temperature).

[00194] Drug product (DP) 10 mg/mL mAb1 is stable when stored at 2-8^{0}C for at least 9 months

[00195] DP 120 mg/mL mAb1 is stable when stored at 2-8^{0}C for at least 9 months.

Example 7: Co-formulation comprising an anti-Ang-2 antibody and a VEGF antagonist

[00196] Co-formulation development activities included the screening of buffers, organic co-solvents, and thermal stabilizers in liquid formulations of VEGF antagonist and anti-Ang-2 antibody to identify excipients that enhance the stability of the protein. Buffer conditions were also examined to determine the optimal pH for maximum protein stability. Results generated from these studies were used to develop a stable, liquid co-formulation suitable for clinical use. The VEGF antagonist consists of a dimer of two polypeptides consisting of amino acids 27-457 of SEQ ID NO: 11 (also referred to herein as aflibercept). The anti-Ang-2 antibody is the human anti-Ang-2 antibody with HCVR/LCVR of SEQ ID NOs: 1/2 and designated as H1H685 in US Patent Application Publication US20110027286 (also referred to herein as “mAb1”). The VEGF antagonist was co-formulated with anti-Ang-2 antibody at four concentrations:

(i) 40 mg/mL ± 6.0 mg/mL aflibercept with 10mg/mL ± 1.5 mg/mL mAb1,

(ii) 40 mg/mL ± 6.0 mg/mL aflibercept with 20 mg/mL ± 3.0 mg/mL mAb1,

(iii) 40 mg/mL ± 6.0 mg/mL aflibercept with 60 mg/mL ± 9.0 mg/mL mAb1, and

(iv) 40 mg/mL ± 6.0 mg/mL aflibercept with 120 mg/mL ± 18.0 mg/mL mAb1.

[00197] In various embodiments, the anti-Ang-2 antibody and VEGF antagonist are co-formulated in 10 ± 1.5 mM sodium phosphate (pH 6.2 ± 0.3), 0.03% ± 0.0045% polysorbate 20, 40 mM ± 6.0 mM sodium chloride, and 5% ± 0.75% sucrose, in water.

[00198] The stability of the formulated drug substance and drug product was assessed using assays as described in Example 8 herein. Charge variant analysis was done using imaged capillary isoelectric focusing (iCIEF) for the formulated drug substance and drug product.

[00199] Stability studies were initiated to determine the storage, accelerated (temperatures above storage conditions), and stress (agitation and freezing and thawing) stability of research lots of 10 mg/mL : 40 mg/mL and 120 mg/mL : 40 mg/mL (mAb1:aflibercept) FDS. These conditions were chosen to bracket the protein concentrations of FDS that used to manufacture the clinical DP. Evaluation of research lots of FDS under accelerated and stress conditions was performed by subjecting FDS to a variety of tests designed to exceed stresses the FDS may encounter during the manufacture of DP and to elucidate the degradation pathways for mAb1:aflibercept FDS. FDS was filled in 5 mL polycarbonate vials.

[00200] The research stability for the co-formulated drug substance was studied as shown in Tables 23 and 24:

[00201] Table 23: Research Stability Study for mAb1:aflibercept Formulated Drug Substance

Storage Stability[1)]		**Container/Closure**
Storage Temperature	**Length of Storage (months)**	5 mL Nalge-Nunc polycarbonate vial with lined closure
-80°C	0, 1, 3, 6, 9, 12, 18, 24, and 36	
-30°C	0, 1, 3, 6, 9, 12, 18, 24, and 36	
-20°C	0, 1, 3, 6, 9, 12, 18, 24, and 36	
Accelerated Stability[2)]		
Incubation Condition	**Length of Incubation**	
5°C	0, 14, 28, and 56 days	
25°C/60% RH	0, 7, 14, and 28 days	
40°C/75% RH	0, 7, 14, and 28 days	
Stress Stability		
Stress	**Duration of Stress**	
Agitation (vortex)	0, 60, and 120 minutes	
Freeze/Thaw[3)]	0, 4, and 8 cycles	

[00202] Table 24: Analysis Plan for mAb1:aflibercept Formulated Drug Substance Research Stability Study

Assay	**Samples to be Analyzed**
Color and Appearance	All Samples
pH	All Samples
Turbidity (Increase in OD at 405 nm)	All Samples
% mAb1 Recovered by RP-HPLC	All Samples
% Aflibercept Recovered by RP-HPLC	All Samples

Assay	Samples to be Analyzed
Total Purity (mAb1 + aflibercept) by Non-Reduced and Reduced SDS-PAGE	t = 0, 6, 12, 24 and 36 months at -80°C, -30°C, and -20°C 56 days at 5°C, 28 days at 25°C/60% RH and 40°C/75% RH °C 120 min Agitation, 8X Freeze/Thaw
Total (mAb1 + aflibercept) Purity by SE-UPLC	All Samples
REGN910 Charge Variant Analysis by iCIEF	All Samples
Aflibercept Charge Variant Analysis by iCIEF	All Samples
% mAb1 Relative Potency byBioassay	t = 0, 6, 12, 24 and 36 months at -80°C and -20°C 56 days at 5°C, 28 days at 25°C/60% RH and 40°C/75% RH °C 120 min Agitation, 8X Freeze/Thaw
% Aflibercept Relative Potency by Bioassay	t = 0, 6, 12, 24 and 36 months at -80°C and -20°C 56 days at 5°C, 28 days at 25°C/60% RH and 40°C/75% RH °C 120 min Agitation, 8X Freeze/Thaw

[00203] The stability results for the co-formulated drug substance are summarized in Tables 25 – 34 below:

[00204] Table 25: Research Stability of mAb1:aflibercept (10 mg/mL: 40 mg/mL) Formulated Drug Substance Stored at -80°C

Formulation			**10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**					
Fill Volume			**1.0 mL**					
Container/Closure			**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
			Length of Storage (months)					
Assay			**0**	**1**	**3**	**6**	**9**	**12**
Color and Appearance			Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)			0.00	0.01	0.00	0.00	0.00	0.00
pH			6.3	6.3	6.3	6.3	6.3	6.3
% Total Recovered by RP-HPLC	mAb1		100	104	104	95	99	94
	Aflibercept		100	102	101	103	99	102
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		98	NR	NR	98	NR	96
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		98	NR	NR	98	NR	98
Purity by SE-UPLC	% Total HMW		2.5	2.5	2.4	2.4	2.4	2.4
	% Total Native		96.8	97.0	96.9	97.4	97.0	97.0
	% Total LMW		0.7	0.6	0.7	0.2	0.6	0.6
Charge Variant Analysis by	mAb1	% Acidic	33.6	34.3	33.6	33.8	35.6	33.9
		% Main	59.9	60.0	59.7	59.7	57.6	59.8

Formulation			10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose					
Fill Volume			**1.0 mL**					
Container/Closure			**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
Assay			**Length of Storage (months)**					
			0	**1**	**3**	**6**	**9**	**12**
iCIEF		% Basic	6.5	5.8	6.7	6.6	6.8	6.3
	Aflibercept	% Acidic	17.4	17.3	17.1	16.8	17.9	16.7
		% Main	78.4	78.6	78.6	78.9	77.8	79.2
		% Basic	4.2	4.2	4.4	4.3	4.4	4.1
% Relative Potency by Bioassay	mAb1		87	NR	NR	123	NR	86
	Aflibercept		118	NR	NR	132	NR	100

[00205] Table 26: Research Stability of mAb1:aflibercept (10 mg/mL: 40 mg/mL) Formulated Drug Substance Stored at -30°C

Formulation		10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
Assay		**Length of Storage (months)**					
		0	**1**	**3**	**6**	**9**	**12**
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.01	0.00	0.00	0.00	0.00
pH		6.3	6.3	6.3	6.3	6.3	6.3
% Total Recovered by RP-HPLC	mAb1	100	105	104	94	102	95
	Aflibercept	100	103	102	103	101	102
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main	98	NR	NR	98	NR	96
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main	98	NR	NR	98	NR	98
Purity by SE-UPLC	% Total HMW	2.5	2.5	2.4	2.5	2.4	2.4
	% Total Native	96.8	96.9	96.9	97.3	97.0	97.2
	% Total LMW	0.7	0.6	0.7	0.2	0.6	0.5

Formulation			**10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**					
Fill Volume			**1.0 mL**					
Container/Closure			**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
			Length of Storage (months)					
Assay			**0**	**1**	**3**	**6**	**9**	**12**
Charge Variant Analysis by iCIEF	mAb1	% Acidic	33.6	34.2	34.4	34.3	33.5	33.6
		% Main	59.9	59.3	59.1	60.1	60.0	60.0
		% Basic	6.5	6.5	6.5	5.6	6.5	6.4
	Aflibercept	% Acidic	17.4	17.0	17.1	17.5	17.1	17.1
		% Main	78.4	78.7	78.8	78.5	78.7	78.8
		% Basic	4.2	4.3	4.2	4.1	4.2	4.1
% Relative Potency by Bioassay	mAb1		87	NR	NR	NR	NR	NR
	Aflibercept		118	NR	NR	NR	NR	NR

[00206] Table 27: Research Stability of mAb1:aflibercept (10 mg/mL: 40 mg/mL) Formulated Drug Substance Stored at -20°C

Formulation		**10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
		Length of Storage (months)					
Assay		**0**	**1**	**3**	**6**	**9**	**12**
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.01	0.00	0.00	0.00	0.00
pH		6.3	6.3	6.3	6.3	6.3	6.3
% Total Recovered by RP-HPLC	mAb1	100	105	101	101	108	98
	Aflibercept	100	103	100	100	103	105
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main	98	NR	NR	98	NR	96
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main	98	NR	NR	96	NR	98
Purity by	% Total HMW	2.5	2.5	2.6	2.8	2.6	2.7

Formulation			**10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**					
Fill Volume			**1.0 mL**					
Container/Closure			**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
			Length of Storage (months)					
Assay			**0**	**1**	**3**	**6**	**9**	**12**
SE-UPLC	% Total Native		96.8	97.0	96.6	97.1	96.8	96.7
	% Total LMW		0.7	0.5	0.7	0.2	0.6	0.6
Charge Variant Analysis by iCIEF	mAb1	% Acidic	33.6	34.5	33.2	34.2	33.8	33.5
		% Main	59.9	59.2	61.0	59.6	59.9	60.1
		% Basic	6.5	6.3	5.8	6.2	6.3	6.4
	Aflibercept	% Acidic	17.4	17.5	17.3	17.4	16.9	17.0
		% Main	78.4	78.5	78.6	78.4	78.9	78.8
		% Basic	4.2	4.0	4.2	4.3	4.2	4.2
% Relative Potency by Bioassay	mAb1		87	NR	NR	98	NR	120
	Aflibercept		118	NR	NR	126	NR	125

[00207] Table 28: Research Stability of mAb1:aflibercept (120 mg/mL: 40 mg/mL) Formulated Drug Substance Stored at -80°C

Formulation		**120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
		Length of Storage (months)					
Assay		**0**	**1**	**3**	**6**	**9**	**12**
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.01	0.01	0.00	0.00	0.00
pH		6.3	6.3	6.3	6.3	6.3	6.3
% Total Recovered by RP-HPLC	mAb1	100	103	99	100	105	104
	Aflibercept	100	98	94	99	96	96
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main	98	NR	NR	98	NR	97

Formulation			**120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**					
Fill Volume			**1.0 mL**					
Container/Closure			**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
Assay			**Length of Storage (months)**					
			0	**1**	**3**	**6**	**9**	**12**
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		99	NR	NR	99	NR	98
Purity by SE-UPLC	% Total HMW		2.8	2.7	2.7	2.7	2.7	2.6
	% Total Native		96.4	96.6	96.4	96.9	96.3	96.9
	% Total LMW		0.8	0.7	1.0	0.4	1.0	0.6
Charge Variant Analysis by iCIEF	mAb1	% Acidic	33.3	33.4	33.6	33.2	33.3	33.6
		% Main	60.4	60.4	60.0	60.5	60.3	60.1
		% Basic	6.3	6.3	6.4	6.3	6.5	6.3
	Aflibercept	% Acidic	15.9	14.7	15.8	15.6	17.3	14.6
		% Main	80.7	81.2	80.0	80.6	78.8	80.9
		% Basic	3.4	4.1	4.2	3.8	3.9	4.4
% Relative Potency by Bioassay	mAb1		70	NR	NR	130	NR	80
	Aflibercept		109	NR	NR	109	NR	84

[00208] Table 29: Research Stability of mAb1:aflibercept (120 mg/mL: 40 mg/mL) Formulated Drug Substance Stored at -30°C

Formulation		**120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
		Length of Storage (months)					
Assay		**0**	**1**	**3**	**6**	**9**	**12**
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.01	0.00	0.00	0.00	0.00
pH		6.3	6.3	6.3	6.3	6.3	6.3
% Total Recovered by RP-HPLC	mAb1	100	105	101	104	104	105
	Aflibercept	100	99	94	101	96	97

Formulation			**120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**					
Fill Volume			**1.0 mL**					
Container/Closure			**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
			Length of Storage (months)					
Assay			**0**	**1**	**3**	**6**	**9**	**12**
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		98	NR	NR	98	NR	97
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		99	NR	NR	99	NR	98
Purity by SE-UPLC	% Total HMW		2.8	2.7	2.7	2.8	2.8	2.7
	% Total Native		96.4	96.6	96.8	96.7	96.3	96.8
	% Total LMW		0.8	0.7	0.6	0.6	1.0	0.6
Charge Variant Analysis by iCIEF	mAb1	% Acidic	33.3	33.5	33.5	33.4	33.2	33.5
		% Main	60.4	60.3	60.1	60.3	60.4	60.3
		% Basic	6.3	6.3	6.3	6.3	6.4	6.2
	Aflibercept	% Acidic	15.9	16.2	16.1	17.3	16.8	14.9
		% Main	80.7	79.4	79.6	78.6	79.3	80.6
		% Basic	3.4	4.4	4.3	4.1	3.9	4.5
% Relative Potency by Bioassay	mAb1		70	NR	NR	NR	NR	NR
	Aflibercept		109	NR	NR	NR	NR	NR

[00209] Table 30: Research Stability of mAb1:aflibercept (120 mg/mL: 40 mg/mL) Formulated Drug Substance Stored at -20°C

Formulation		**120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**					
Fill Volume		**1.0 mL**					
Container/Closure		**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
		Length of Storage (months)					
Assay		**0**	**1**	**3**	**6**	**9**	**12**
Color and Appearance		Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.01	0.00	0.00	0.00	0.00
pH		6.3	6.3	6.3	6.3	6.3	6.3
% Total Recovered	mAb1	100	104	103	103	106	107

Formulation			**120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**					
Fill Volume			**1.0 mL**					
Container/Closure			**5 mL polycarbonate vial with silicone lined polypropylene screw cap**					
			Length of Storage (months)					
Assay			**0**	**1**	**3**	**6**	**9**	**12**
by RP-HPLC	Aflibercept		100	99	96	101	97	99
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		98	NR	NR	97	NR	97
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		99	NR	NR	99	NR	97
Purity by SE-UPLC	% Total HMW		2.8	2.7	2.7	2.8	2.9	2.8
	% Total Native		96.4	96.7	96.6	96.7	96.2	96.7
	% Total LMW		0.8	0.6	0.8	0.6	0.9	0.6
Charge Variant Analysis by iCIEF	mAb1	% Acidic	33.3	34.2	34.1	33.9	34.7	33.3
		% Main	60.4	59.6	59.6	59.8	59.1	60.4
		% Basic	6.3	6.2	6.4	6.3	6.2	6.3
	Aflibercept	% Acidic	15.9	16.4	14.3	17.2	15.5	16.7
		% Main	80.7	80.0	81.3	78.0	79.5	79.1
		% Basic	3.4	3.7	4.4	4.8	5.1	4.2
% Relative Potency by Bioassay	mAb1		70	NR	NR	83	NR	110
	Aflibercept		109	NR	NR	133	NR	140

[00210] Table 31: Research Stability of mAb1:aflibercept (10 mg/mL : 40 mg/mL) Formulated Drug Substance – Effect of Accelerated Conditions

Formulation	**10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**									
Fill Volume	**1.0 mL**									
Container/Closure	**5 mL polycarbonate vial w/ silicone lined polypropylene screw cap**									
	Storage Condition/Length of Storage (days)									
	No Storage	**5°C**			**25°C/60%RH**			**40°C/75%RH**		
Assay	**0**	**14**	**28**	**56**	**7**	**14**	**28**	**7**	**14**	**28**
Color and Appearance	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
pH	6.3	6.3	6.3	6.4	6.3	6.3	6.3	6.3	6.3	6.2

Formulation			10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose									
Fill Volume			1.0 mL									
Container/Closure			5 mL polycarbonate vial w/ silicone lined polypropylene screw cap									
			Storage Condition/Length of Storage (days)									
			No Storage	5°C			25°C/60%RH			40°C/75%RH		
Assay			0	14	28	56	7	14	28	7	14	28
% Total Recovered by RP-HPLC	mAb1		100	97	105	102	98	102	113	101	103	115
	Aflibercept		100	103	105	106	105	108	109	108	110	114[1)]
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		98	NR	NR	97	NR	NR	98	NR	NR	95
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		98	NR	NR	98	NR	NR	97	NR	NR	96
Purity by SE-UPLC	% Total HMW		2.5	2.2	2.3	2.3	2.2	2.6	2.8	4.8	9.5	11.2
	% Total Native		96.8	96.6	97.1	97.6	96.8	96.3	96.6	94.2	88.8	87.9
	% Total LMW		0.7	1.2	0.6	0.2	1.0	1.2	0.7	1.0	1.7	0.9
Charge Variant Analysis by iCIEF	mAb1	% Acidic	33.6	34.2	34.2	34.3	34.3	36.3	36.3	38.7	49.6	53.5
		% Main	59.9	59.3	59.3	59.5	59.8	56.9	56.7	53.6	42.0	38.3
		% Basic	6.5	6.5	6.5	6.2	6.0	6.8	7.0	7.7	8.4	8.2
	Aflibercept	% Acidic	17.4	17.6	17.6	16.9	17.2	17.3	18.1	18.3	21.9	23.4
		% Main	78.4	78.0	78.0	78.8	78.6	78.5	77.8	77.8	75.5	73.8
		% Basic	4.2	4.4	4.4	4.3	4.2	4.2	4.2	3.9	2.7	2.8
% Relative Potency by Bioassay	mAb1		87	NR	NR	97	NR	NR	113	NR	NR	136
	Aflibercept		118	NR	NR	109	NR	NR	71	NR	NR	112

[00211] Table 32: Research Stability of mAb1:aflibercept (120 mg/mL: 40 mg/mL) Formulated Drug Substance – Effect of Accelerated Conditions

Formulation	120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose									
Fill Volume	1.0 mL									
Container/Closure	5 mL polycarbonate vial w/ silicone lined polypropylene screw cap									
	Storage Condition/Length of Storage (days)									
	No Storage	5°C			25°C/60%RH			40°C/75%RH		
Assay	0	14	28	56	7	14	28	7	14	28
Color and Appearance	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.01
pH	6.3	6.3	6.3	6.4	6.3	6.3	6.2	6.3	6.3	6.2

Formulation			120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose									
Fill Volume			1.0 mL									
Container/Closure			5 mL polycarbonate vial w/ silicone lined polypropylene screw cap									
			Storage Condition/Length of Storage (days)									
			No Storage	5°C			25°C/60%RH			40°C/75%RH		
Assay			0	14	28	56	7	14	28	7	14	28
% Total Recovered by RP-HPLC	mAb1		100	99	103	100	102	102	110	102	104	126
	Aflibercept		100	101	102	101	103	103	107	103	104	118[1)]
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		98	NR	NR	95	NR	NR	97	NR	NR	94
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		99	NR	NR	99	NR	NR	99	NR	NR	97
Purity by SE-UPLC	% Total HMW		2.8	2.8	2.9	3.0	3.0	3.4	3.5	5.1	8.6	10.7
	% Total Native		96.4	96.0	96.4	96.4	96.1	95.3	95.5	93.8	89.2	87.0
	% Total LMW		0.8	1.1	0.7	0.6	0.9	1.3	0.9	1.1	2.3	2.3
Charge Variant Analysis by iCIEF	REGN 910	% Acidic	33.3	33.5	33.6	33.5	34.2	35.8	36.2	38.7	48.9	51.9
		% Main	60.4	60.1	60.1	60.0	59.1	57.4	56.8	53.4	42.0	38.7
		% Basic	6.3	6.4	6.3	6.4	6.7	6.9	7.0	7.9	9.1	9.4
	Aflibercept	% Acidic	15.9	15.8	15.7	15.8	15.9	16.4	16.9	14.4	19.8	22.8
		% Main	80.7	80.3	79.9	80.0	79.6	79.4	78.9	81.7	77.1	75.0
		% Basic	3.4	3.9	4.3	4.2	4.5	4.2	4.2	3.9	3.1	2.2
% Relative Potency by Bioassay	mAb1		70	NR	NR	97	NR	NR	141	NR	NR	143
	Aflibercept		109	NR	NR	121	NR	NR	107	NR	NR	104

[00212] Table 33: Research Stability of mAb1:aflibercept (10 mg/mL : 40 mg/mL) Formulated Drug Substance – Effect of Stress Conditions

Formulation	10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose				
Fill Volume	1.0 mL				
Container/Closure	5 mL polycarbonate vial w/ silicone lined polypropylene screw cap				
	Stress Condition/Length of Stress				
	No Stress	Agitation (minutes)		Freeze/Thaw (Cycles)	
Assay	0	60	120	4	8
Color and Appearance	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)	0.00	0.00	0.00	0.00	0.00

Formulation			10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose				
Fill Volume			**1.0 mL**				
Container/Closure			**5 mL polycarbonate vial w/ silicone lined polypropylene screw cap**				
			Stress Condition/Length of Stress				
			No Stress	**Agitation (minutes)**		**Freeze/Thaw (Cycles)**	
Assay			**0**	**60**	**120**	**4**	**8**
pH			6.3	6.3	6.3	6.3	6.2
% Total Recovered by RP-HPLC	mAb1		100	99	99	105	104
	Aflibercept		100	103	103	102	102
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		98	NR	98	NR	98
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		98	NR	98	NR	98
Purity by SE-UPLC	% Total HMW		2.5	2.4	2.3	2.5	2.4
	% Total Native		96.8	96.8	97.0	97.0	97.0
	% Total LMW		0.7	0.8	0.7	0.5	0.6
Charge Variant Analysis by iCIEF	mAb1	% Acidic	33.6	34.3	34.5	34.7	35.0
		% Main	59.9	59.6	59.3	59.4	58.8
		% Basic	6.5	6.1	6.2	5.9	6.2
	Afliber cept	% Acidic	17.4	17.9	17.9	17.8	17.9
		% Main	78.4	77.9	77.8	78.1	77.8
		% Basic	4.2	4.2	4.3	4.1	4.3
% Relative Potency by Bioassay	mAb1		87	NR	96	NR	113
	Aflibercept		118	NR	104	NR	133

[00213] Table 34: Research Stability of mAb1:aflibercept (120 mg/mL : 40 mg/mL) Formulated Drug Substance – Effect of Stress Conditions

Formulation	**120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**
Fill Volume	**1.0 mL**
Container/Closure	**5 mL polycarbonate vial w/ silicone lined polypropylene screw cap**

Assay			Stress Condition/Length of Stress				
			No Stress	Agitation (minutes)		Freeze/Thaw (Cycles)	
			0	60	120	4	8
Color and Appearance			Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)			0.00	0.00	0.00	0.01	0.01
pH			6.3	6.3	6.3	6.3	6.3
% Total Recovered by RP-HPLC	mAb1		100	99	101	104	103
	Aflibercept		100	98	101	98	98
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		98	NR	98	NR	98
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		99	NR	99	NR	99
Purity by SE-UPLC	% Total HMW		2.8	3.2	3.7	2.7	2.8
	% Total Native		96.4	96.3	95.7	96.5	96.4
	% Total LMW		0.8	0.6	0.6	0.8	0.8
Charge Variant Analysis by iCIEF	mAb 1	% Acidic	33.3	34.3	34.2	33.9	34.0
		% Main	60.4	59.4	59.4	59.8	59.6
		% Basic	6.3	6.2	6.4	6.4	6.3
	Aflibercept	% Acidic	15.9	16.8	16.3	15.8	16.6
		% Main	80.7	78.3	79.0	79.9	79.3
		% Basic	3.4	4.9	4.7	4.3	4.2
% Relative Potency by Bioassay	mAb1		70	NR	97	NR	75
	Aflibercept		109	NR	95	NR	143

[00214] The stability of the co-formulated drug products was studied as shown in Tables 35 and 36:

[00215] Table 35: Research Stability Studies for mAb1:aflibercept Drug Products

Storage Stability[1)]		**Container/Closure**
Storage Temperature	**Length of Storage (months)**	Type 1 borosilicate glass with FluroTec® coated 4432/50 butyl rubber stopper
5°C	0, 1, 3, 6, 9, 12, 18, 24, and 36	
Accelerated Stability[2)]		
Incubation Condition	**Length of Incubation**	
25°C	0, 1, 3, and 6 months	
37°C	0, 7, 14, and 28 days	
Stress Stability		
Stress	**Duration of Stress**	

Storage Stability[1)]		Container/Closure
Agitation (vortex)	0, 60, and 120 minutes	
Freeze/Thaw[3)]	0, 4, and 8 cycles	

[00216] Table 36: Research Stability Studies Analysis Plan for mAb1:aflibercept Drug Products

Assay	Samples to be Analyzed
Color and Appearance	All Samples
pH	All Samples
Turbidity (Increase in OD at 405 nm)	All Samples
% Total mAb1 Recovered by RP-HPLC	All Samples
% Aflibercept Recovered by RP-HPLC	All Samples
Total Purity (mAb1 + aflibercept) by Non-Reduced and Reduced SDS-PAGE	t = 0, 6, 12, 24 and 36 months at 5°C; 6 months at 25°C; 28 days at 37°C 120 min Agitation, 8X Freeze/Thaw
Total (mAb1 + aflibercept) Purity by SE-UPLC	All Samples
mAb1 Charge Variant Analysis by iCIEF	All Samples
Aflibercept Charge Variant Analysis by iCIEF	All Samples
Particulate Matter Analysis by MFI	t = 0, 6, 12, 24 and 36 months at 5°C; 6 months at 25°C; 28 days at 37°C 120 min Agitation, 8X Freeze/Thaw
% mAb1 Relative Potency by Bioassay	t = 0, 6, 12, 24 and 36 months at 5°C; 6 months at 25°C; 28 days at 37°C 120 min Agitation, 8X Freeze/Thaw
% Aflibercept Relative Potency by Bioassay	t = 0, 6, 12, 24 and 36 months at 5°C; 6 months at 25°C; 28 days at 37°C 120 min Agitation, 8X Freeze/Thaw

[00217] The results of the stability for the co-formulated drug products are summarized in Tables 37 – 42 below:

[00218] Table 37: Research Stability of mAb1:aflibercept (10 mg/mL : 40 mg/mL) Drug Product Stored at 5℃

Formulation		**10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**				
Fill Volume		**0.5 mL**				
Container/Closure		**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**				
Assay		**Length of Storage (months)**				
		0	**1**	**3**	**6**	**9**
Color and Appearance		Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)		0.00	0.00	0.00	0.00	0.00
pH		6.3	6.3	6.3	6.3	6.3
% Total Recovered	mAb1	100	100	95	104	94

Formulation			**10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**				
Fill Volume			**0.5 mL**				
Container/Closure			**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**				
Assay			**Length of Storage (months)**				
			0	**1**	**3**	**6**	**9**
by RP-HPLC	Aflibercept		100	102	101	97	97
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		99	NR	NR	98	NR
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		97	NR	NR	98	NR
Purity by SE-UPLC	% Total HMW		1.6	1.7	1.8	1.9	2.0
	% Total Native		97.6	97.8	97.8	97.4	97.5
	% Total LMW		0.8	0.6	0.4	0.7	0.5
Charge Variant Analysis by iCIEF	mAb1	% Acidic	37.1	37.6	37.5	37.5	37.6
		% Main	58.1	58.0	57.8	58.2	57.8
		% Basic	4.8	4.4	4.7	4.3	4.7
	Aflibercept	% Acidic	19.5	19.7	19.7	20.3	19.4
		% Main	77.5	77.0	76.3	76.7	77.7
		% Basic	3.0	3.2	4.0	3.0	2.9
Particulate Analysis by MFI (particles/mL)	≥ 2 μm		4137	NR	NR	2344	NR
	≥ 10 μm		103	NR	NR	23	NR
	≥ 25 μm		15	NR	NR	0	NR
% Relative Potency (Bioassay)	mAb1		124	NR	NR	78	124
	Aflibercept		125	NR	NR	165	107

[00219] Table 38: Research Stability of mAb1:aflibercept (120 mg/mL : 40 mg/mL) Drug Product Stored at 5℃

Formulation	**120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**				
Fill Volume	**0.5 mL**				
Container/Closure	**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**				
Assay	**Length of Storage (months)**				
	0	**1**	**3**	**6**	**9**
Color and Appearance	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)	0.00	0.00	0.00	0.00	0.00
pH	6.3	6.4	6.3	6.3	6.3

Formulation			120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose				
Fill Volume			**0.5 mL**				
Container/Closure			**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**				
Assay			**Length of Storage (months)**				
			0	**1**	**3**	**6**	**9**
% Total Recovered by RP-HPLC	mAb1		100	102	101	103	102
	Aflibercept		100	103	104	98	99
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		97	NR	NR	96	NR
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		95	NR	NR	96	NR
Purity by SE-UPLC	% Total HMW		2.4	2.8	3.0	3.2	3.4
	% Total Native		96.6	96.5	96.3	96.1	96.0
	% Total LMW		1.1	0.7	0.6	0.8	0.7
Charge Variant Analysis by iCIEF	mAb1	% Acidic	37.7	37.6	37.5	37.7	37.5
		% Main	57.8	57.9	57.8	57.6	57.7
		% Basic	4.5	4.5	4.7	4.7	4.8
	Aflibercept	% Acidic	19.5	18.0	18.2	18.8	18.1
		% Main	77.9	80.0	79.1	77.5	77.8
		% Basic	2.6	2.0	2.7	3.7	4.0
Particulate Analysis by MFI (particles/mL)	≥ 2 μm		3276	NR	NR	2400	NR
	≥ 10 μm		145	NR	NR	99	NR
	≥ 25 μm		15	NR	NR	7	NR
% Relative Potency (Bioassay)	mAb1		87	NR	NR	57	76
	Aflibercept		109	NR	NR	130	63

[00220] Table 39: Research Stability of mAb1:aflibercept (10 mg/mL : 40 mg/mL) Drug Product – Effect of Accelerated Conditions

Formulation	10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose						
Fill Volume	**0.5 mL**						
Container/Closure	**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**						
Assay	**Storage Condition/Length of Storage**						
	No Storage	**25°C (months)**			**37°C (days)**		
	0	**1**	**3**	**6**	**7**	**14**	**28**
Color and Appearance	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pH	6.3	6.3	6.4	6.3	6.3	6.3	6.3

Formulation			10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose						
Fill Volume			0.5 mL						
Container/Closure			2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper						
			Storage Condition/Length of Storage						
			No Storage	25°C (months)			37°C (days)		
Assay			0	1	3	6	7	14	28
% Total Recovered by RP-HPLC	mAb1		100	104	99	102	105	103	107
	Aflibercept		100	103	103	98	101	98	101
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		99	NR	NR	94	NR	NR	95
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		97	NR	NR	94	NR	NR	96
Purity by SE-UPLC	% Total HMW		1.6	2.3	2.8	3.5	2.8	3.7	5.3
	% Total Native		97.6	97.1	96.5	95.0	95.9	95.6	93.7
	% Total LMW		0.8	0.7	0.7	1.5	1.3	0.8	1.0
Charge Variant Analysis by iCIEF	mAb1	% Acidic	37.1	39.3	45.9	53.2	40.6	43.7	50.3
		% Main	58.1	54.9	48.2	40.5	53.7	50.2	42.7
		% Basic	4.8	5.8	5.9	6.2	5.7	6.1	7.0
	Afliber cept	% Acidic	19.5	21.3	22.2	26.1	20.9	22.3	24.2
		% Main	77.5	75.5	74.7	71.4	76.0	74.7	72.8
		% Basic	3.0	3.2	3.1	2.6	3.1	3.0	2.9
Particulate Analysis by MFI (particles/mL)	≥ 2 μm		4137	NR	NR	3628	NR	NR	1593
	≥ 10 μm		103	NR	NR	105	NR	NR	61
	≥ 25 μm		15	NR	NR	17	NR	NR	5
% Relative Potency by Bioassay	mAb1		124	NR	NR	59	NR	NR	123
	Aflibercept		125	NR	NR	146	NR	NR	139

[00221] Table 40: Research Stability of mAb1:aflibercept (120 mg/mL : 40 mg/mL) Drug Product – Effect of Accelerated Conditions

Formulation	120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose						
Fill Volume	0.5 mL						
Container/Closure	2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper						
	Storage Condition/Length of Storage						
	No Storage	25°C (months)			37°C (days)		
Assay	0	1	3	6	7	14	28
Color and Appearance	Pass	Pass	Pass	Pass	Pass	Pass	Pass

Formulation			120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose						
Fill Volume			0.5 mL						
Container/Closure			2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper						
			Storage Condition/Length of Storage						
			No Storage	25°C (months)			37°C (days)		
Assay			0	1	3	6	7	14	28
Turbidity (Increase in OD at 405 nm)			0.00	0.00	0.00	0.01	0.00	0.00	0.00
pH			6.3	6.4	6.4	6.3	6.3	6.3	6.4
% Total Recovered by RP-HPLC	mAb1		100	101	101	103	102	99	103
	Aflibercept		100	102	102	97	100	95	99
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		97	NR	NR	91	NR	NR	92
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		95	NR	NR	95	NR	NR	96
Purity by SE-UPLC	% Total HMW		2.4	3.7	4.5	5.2	4.2	5.0	6.5
	% Total Native		96.6	95.5	94.5	93.1	94.5	93.8	92.0
	% Total LMW		1.1	0.8	1.1	1.7	1.4	1.2	1.6
Charge Variant Analysis by iCIEF	mAb1	% Acidic	37.7	40.2	46.4	53.7	41.2	44.5	50.3
		% Main	57.8	54.2	47.5	39.2	52.9	49.2	42.5
		% Basic	4.5	5.6	6.2	7.1	5.9	6.3	7.2
	Afliber cept	% Acidic	19.5	18.8	20.5	23.1	20.2	20.4	22.5
		% Main	77.9	79.0	77.7	75.0	77.7	77.1	75.5
		% Basic	2.6	2.2	1.8	1.9	2.1	2.6	2.0
Particulate Analysis by MFI (particles/mL)	≥ 2 µm		3276	NR	NR	2701	NR	NR	953
	≥ 10 µm		145	NR	NR	55	NR	NR	38
	≥ 25 µm		15	NR	NR	3	NR	NR	3
% Relative Potency by Bioassay	mAb1		87	NR	NR	70	NR	NR	116
	Aflibercept		109	NR	NR	108	NR	NR	79

[00222] Table 41: Research Stability of mAb1:aflibercept (10 mg/mL : 40 mg/mL) Drug Product – Effect of Stress Conditions

Formulation	10 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose
Fill Volume	0.5 mL
Container/Closure	2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper

Assay			Stress Condition/Length of Stress				
			No Stress	Agitation (minutes)		Freeze/Thaw (Cycles)	
			0	60	120	4	8
Color and Appearance			Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)			0.00	0.00	0.00	0.00	0.00
pH			6.3	6.3	6.3	6.3	6.4
% Total Recovered by RP-HPLC	mAb1		100	94	95	99	98
	Aflibercept		100	99	99	101	101
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		99	NR	98	NR	98
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		97	NR	98	NR	98
Purity by SE-UPLC	% Total HMW		1.6	1.5	1.5	1.6	1.6
	% Total Native		97.6	97.7	97.6	97.9	97.8
	% Total LMW		0.8	0.9	0.8	0.5	0.6
Charge Variant Analysis by iCIEF	mAb1	% Acidic	37.1	36.8	37.1	37.6	37.1
		% Main	58.1	58.5	58.7	58.3	58.6
		% Basic	4.8	4.7	4.2	4.1	4.3
	Aflibercept	% Acidic	19.5	19.6	20.0	20.3	20.1
		% Main	77.5	77.3	76.8	76.5	76.7
		% Basic	3.0	3.1	3.2	3.2	3.2
Particulate Analysis by MFI (particles/mL)	≥ 2 µm		4137	NR	3181	NR	2799
	≥ 10 µm		103	NR	164	NR	55
	≥ 25 µm		15	NR	21	NR	5
% Relative Potency (Bioassay)	mAb1		124	NR	122	NR	130
	Aflibercept		125	NR	131	NR	136

[00223] Table 42: Research Stability of mAb1:aflibercept (120 mg/mL : 40 mg/mL) Drug Product – Effect of Stress Conditions

Formulation	**120 mg/mL mAb1, 40 mg/mL aflibercept, 10 mM sodium phosphate, pH 6.2, 40 mM sodium chloride, 0.03% (w/v) polysorbate 20, 5% (w/v) sucrose**
Fill Volume	**0.5 mL**
Container/Closure	**2 mL Type I borosilicate glass vial with West S2-F451 4432/50 GRY B2-40 stopper**

Assay			Stress Condition/Length of Stress				
			No Stress	Agitation (minutes)		Freeze/Thaw (Cycles)	
			0	60	120	4	8
Color and Appearance			Pass	Pass	Pass	Pass	Pass
Turbidity (Increase in OD at 405 nm)			0.00	0.00	0.00	0.00	0.00
pH			6.3	6.3	6.3	6.3	6.4
% Total Recovered by RP-HPLC	mAb1		100	102	101	101	101
	Aflibercept		100	100	100	103	103
Purity by SDS-PAGE	Non-reduced; % mAb1 main + % Aflibercept main		97	NR	97	NR	97
	Reduced; % mAb1 heavy chain + % mAb1 light chain + % Aflibercept main		95	NR	98	NR	98
Purity by SE-UPLC	% Total HMW		2.4	2.3	2.3	2.5	2.6
	% Total Native		96.6	96.9	97.0	96.7	96.7
	% Total LMW		1.1	0.8	0.7	0.8	0.8
Charge Variant Analysis by iCIEF	mAb1	% Acidic	37.7	37.3	37.5	37.5	37.6
		% Main	57.8	58.2	57.9	57.9	57.9
		% Basic	4.5	4.5	4.5	4.6	4.6
	Aflibercept	% Acidic	19.5	19.7	19.7	18.9	19.3
		% Main	77.9	78.1	77.7	78.9	78.3
		% Basic	2.6	2.2	2.6	2.3	2.4
Particulate Analysis by MFI (particles/mL)	≥ 2 µm		3276	NR	5403	NR	6448
	≥ 10 µm		145	NR	216	NR	189
	≥ 25 µm		15	NR	11	NR	11
% Relative Potency (Bioassay)	mAb1		87	NR	108	NR	57
	Aflibercept		109	NR	114	NR	111

[00224] The results of the stability studies indicated that:

[00225] Formulated drug substance (FDS) 10 :40 mg/mL mAb1: aflibercept is stable when stored at ≤ -20^{0}C for at least 12 months

[00226] FDS 120 : 40 mg/mL mAb1:aflibercept is stable when stored at ≤ -20^{0}C for at least 12 months

[00227] 10 mg/mL : 40 mg/mL mAb1:aflibercept FDS was physically and chemically stable after 56 days of incubation at 5℃ or 28 days of incubation at 25℃/60% RH. No appreciable change in the physical or chemical stability was detected in any of the monitored attributes. Both mAb1 and

aflibercept maintained potency when the 10 mg/mL : 40 mg/mL FDS was incubated at 5℃ for 56 days or at 25℃/60% RH for 28 days.

[00228] 120 mg/mL : 40 mg/mL mAb1:aflibercept FDS was physically and chemically stable after 56 days of incubation at 5℃. No appreciable change in the physical or chemical stability was detected in any of the monitored attributes. 120 mg/mL : 40 mg/mL FDS was physically stable after 28 days of incubation at 25℃/60% RH. No appreciable change in the physical or chemical stability was detected in any of the other monitored attributes. mAb1 and aflibercept both maintained potency when the 120 mg/mL : 40 mg/mL FDS was incubated at 5℃ for 56 days or at 25℃/60% RH for 28 days.

[00229] 10 mg/mL : 40 mg/mL mAb1:aflibercept FDS was physically and chemically stable when agitated (vortexed at ambient temperature) for 120 minutes or when subjected to 8 freeze/thaw cycles (freezing at -30°C and thawing at room temperature). No appreciable change in the physical or chemical stability was detected in any of the monitored attributes. mAb1 and aflibercept maintained potency when the 10 mg/mL : 40 mg/mL FDS was agitated (vortexed at ambient temperature) for 120 minutes or when subjected to 8 freeze/thaw cycles (freezing at -30°C and thawing at room temperature).

[00230] 10 mg/mL : 40 mg/mL mAb1:aflibercept FDS was physically and chemically stable when agitated (vortexed at ambient temperature) for 60 minutes or when subjected to 8 freeze/thaw cycles (freezing at -30°C and thawing at room temperature). No appreciable change in the physical or chemical stability was detected in any of the monitored attributes. Although a 0.9% increase in high molecular weight species (SE-UPLC) was observed when FDS was agitated (vortexed at ambient temperature) for 120 minutes, no appreciable change was detected in any of the other monitored attributes. 120 minutes of vortex agitation is an extreme stress and agitation will be minimized for 120 mg/mL : 40 mg/mL mAb1:aflibercept FDS during the manufacturing process. mAb1 and aflibercept maintained potency when the 10 mg/mL : 40 mg/mL mAb1:aflibercept FDS was agitated (vortexed at ambient temperature) for 120 minutes or when subjected to 8 freeze/thaw cycles (freezing at -30°C and thawing at room temperature).

[00231] Drug product (DP) 10 : 40 mg/mL mAb1:aflibercept is stable when stored at 2-8^{0}C for at least 9 months

[00232] DP 120 : 40 mg/mL mAb1:aflibercept is stable when stored at 2-8^{0}C for at least 9 months.

Example 8: Methods used to assess stability of formulations

[00233] The research stability of mAb1 and mAb1:aflibercept FDS and DP were assessed using the following assays: Color and appearance by visual inspection; pH; Turbidity measured by increase in Optical Density (OD) at 405 nm; Subvisible particulate analysis on DP by Microflow

Imaging (MFI); Protein concentration by reversed-phase high performance liquid chromatography (RP-HPLC).

[00234] Purity was assessed by the following assays: Size exclusion ultra performance liquid chromatography (SE-UPLC); Reduced and non-reduced sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

[00235] Charge variant analysis was assessed by the following assays: Cation exchange UPLC (CEX-UPLC) for mAb1 FDS and DP; imaged capillary isoelectric focusing (iCIEF) for mAb1 and mAb1:aflibercept FDS and DP.

[00236] Potency was assessed by bioassays performed for mAb1 in the mAb1 formulation; and for both mAb1 and aflibercept in the mAb1:aflibercept formulation. The relative potency of each sample is determined using a bioassay and is defined as: (IC_{50} Reference Sample/IC_{50} Sample)*100%. The measured potency of storage stability samples must be within 50-150% of the measured potency of the reference standard.

[00237] The physical stability of a formulation refers to properties such as color, appearance, pH, turbidity and protein concentration. The presence of visible particulates in solution can be detected by visual inspection. A solution passes visual inspection if it is clear to slightly opalescent, essentially free from visible particulates, and colorless to pale yellow. Turbidity, measured by an increase in OD at 405 nm, can also be used to detect particulates in solution. An increase in OD at 405 nm may indicate the presence of particulates, an increase in opalescence, or color change of the test articles. MFI is used to measure subvisible particulates that are ≥2 µm in size. Protein concentration is measured by a RP-HPLC assay and reported as percent protein recovery relative to the starting material. In the RP-HPLC assay, a single mAb1 peak is resolved from a single aflibercept peak following elution from the reversed phase column. The mAb1 concentration is determined by comparing the mAb1 peak area to a calibration curve generated using a mAb1 standard whereas the aflibercept concentration is determined by comparing the aflibercept peak area to a calibration curve generated using an aflibercept standard. Percent recovery is calculated based on the measured mAb1 or aflibercept concentration relative to the starting mAb1 or aflibercept concentration, respectively.

[00238] Chemical stability refers to the formation of covalently modified forms (e.g. covalent aggregates, cleavage products or charge variant forms) and non-covalently modified forms (e.g. non-covalent aggregates) of protein. Higher and lower molecular weight degradation products can be separated from native molecular weight product using SE-UPLC and SDS-PAGE methods. The percentage of degraded mAb1 in the SE-UPLC method is calculated from the ratio of the area of all non-native peaks to the total area of all mAb1 peaks. mAb1 Purity by non-reduced and reduced SDS-PAGE is calculated from the ratio of main band intensity to the total intensity of all

bands. Charge variant forms of mAb1 are resolved using iCIEF and CEX-UPLC. In these methods, peaks that are focused to a pI lower than that of the main peak are labeled “Acidic” peaks, whereas those focused to a pI higher than that of the main peak are labeled “Basic” peaks.

[00239] mAb1:aflibercept formulations are characterized using total molecular weight purity (native mAb1 + native aflibercept) by SE-UPLC (i.e. molecular weight purity of mAb1 and aflibercept will not be determined individually), because mAb1 and aflibercept native species cannot be fully resolved from each other. Similarly, mAb1:aflibercept formulations will be characterized using total HMW species (mAb1 HMW + aflibercept HMW) and total LMW species (mAb1 LMW + aflibercept LMW), because mAb1 HMW species cannot be resolved from aflibercept HMW species and mAb1 LMW species cannot be resolved from aflibercept LMW species.

[00240] The percentage of total HMW species or total LMW species in mAb1:aflibercept, determined using the SE-UPLC method is calculated from the ratio of the area of total HMW species or total LMW species to the total area of all mAb1:aflibercept peaks, respectively. Purity by non-reduced and reduced SDS-PAGE is calculated from the ratio of (aflibercept main band + mAb1 main band) intensity to the total intensity of all bands. Aflibercept is a highly glycosylated protein that contains a high level of sialic acid. As a result of the complex charge distribution on aflibercept the CEX-UPLC method did not have sufficient resolution to separate all charge variant forms and was therefore not used to assay changes in the charge variant profile for the mAb1:aflibercept samples. Charge variant forms of mAb1 and aflibercept that are co-formulated within mAb1:aflibercept were resolved using iCIEF only.

[00241] For mAb1, peaks that are focused to a pI lower than that of the main peak are labeled “Acidic” peaks, whereas those focused to a pI higher than that of the main peak are labeled “Basic” peaks.. For aflibercept, peaks 3-8 are the major peaks and are labeled “Main” peaks. Aflibercept peaks that are focused to a pI lower than that of peak 3 are labeled “Acidic” peaks, whereas those focused to a pI higher than that of peak 8 are labeled “Basic” peaks.

Example 9: Storage stability comparison of mAb1, mAb1:aflibercept and aflibercept formulations

[00242] SE-UPLC results from studies evaluating the storage stability of research formulations of 10 mg/mL mAb1, 120 mg/mL mAb1, and 40 mg/mL aflibercept were compared to results from the mAb1:aflibercept (10 mg/mL : 40 mg/mL) and (120 mg/mL : 40 mg/ml) co-formulations. This evaluation was performed to determine if co-formulating mAb1 and aflibercept resulted in differences in the relative amounts of high molecular weight species formed when compared to the individually formulated solutions of mAb1 and aflibercept (mono-formulations). The SE-UPLC results from the mono-formulations, incubated under equivalent conditions as the co-formulations,

were used to calculate a theoretical increase in % HMW species for a co-formulation containing the same concentrations of each protein. The theoretical increase in HMW was compared to the increase in HMW observed when the actual co-formulated drug product was incubated at 5°C. The DS lots used for these studies are representative of the DS manufactured for clinical use.

[00243] A summary of the SE-UPLC results for formulations stored at 5°C containing 10 mg/mL mAb1 alone, 40 mg/mL aflibercept alone, and the mAb1:aflibercept (10 mg/mL : 40 mg/mL) co-formulation is as follows: After 9 months of storage at 5°C, the HMW content in the 10 mg/mL mAb1 formulation did not increase. Over the same assessment period the % HMW in the 40 mg/mL aflibercept formulation increased 0.4%. Based on the results from the 10 mg/mL mAb1 and 40 mg/mL aflibercept formulations an increase of 0.4% HMW species was predicted to occur over the 9 month assessment period in a mAb1:aflibercept (10 mg/mL : 40 mg/mL) co-formulation. A 0.4% increase in total HMW species was observed for the mAb1:aflibercept (10 mg/mL : 40 mg/mL) DP during the 9 month assessment period. The agreement between the calculated and observed increase in %HMW suggest that storage of mAb1 and aflibercept as mAb1:aflibercept (10 mg/mL : 40 mg/mL) DP for 9 months at 5°C did not lead to enhanced generation of HMW forms, relative to the increases observed for the individually formulated drug products.

[00244] A summary of the SE-UPLC results for formulations stored at 5°C containing 120 mg/mL mAb1 alone, 40 mg/mL aflibercept alone, and the mAb1:aflibercept (120 mg/mL : 40 mg/mL) co-formulation is as follows: After 9 months of storage at 5°C, the HMW content in the 120 mg/mL mAb1 formulation and the 40 mg/mL aflibercept formulations increased by 0.5% and 0.4%, respectively. Based on the results from the 120 mg/mL mAb1 and 40 mg/mL aflibercept formulation an increase of 0.9% HMW species was predicted to occur over the 9 month incubation period in a mAb1:aflibercept (120 mg/mL : 40 mg/mL) co-formulation. This theoretical result was comparable to the 1.0% increase in total HMW species observed for the mAb1:aflibercept (120 mg/mL : 40 mg/mL) DP during 9 months of storage at 5°C. The close agreement between the calculated and actual increases in %HMW suggest that storage of mAb1 and aflibercept as mAb1:aflibercept (120 mg/mL : 40 mg/mL) DP for 9 months at 5°C did not lead to enhanced generation of HMW forms, relative to the increases observed for the individually formulated drug products.

Example 10: Clinical trial of intravitreally (IVT) administered mAb1 in combination with aflibercept in patients with either neo-vascular AMD or DME

[00245] This study is a phase I, open-label, dose escalation clinical study designed to evaluate the safety, tolerability, and efficacy of IVT administration of mAb1 alone or in combination with aflibercept in patients with neovascular AMD or DME.

[00246] The primary objective of the study is to investigate the safety and tolerability of IVT mAb1 and aflibercept, and IVT mAb1 in patients with neovascular AMD, and separately in patients with DME.

[00247] The secondary objectives of the study are: (i) to characterize the systemic pharmacokinetics (PK) of mAb1 and aflibercept following IVT injection of mAb1 and aflibercept; and (ii) to characterize the presence of anti-mAb1 and anti-aflibercept antibodies following IVT injection.

[00248] The primary endpoint is the incidence and severity of ocular and systemic treatment-emergent adverse events (TEAEs) through week 24 in patients treated with IVT mAb1 alone or with IVT co-formulated mAb1 and aflibercept.

[00249] The secondary endpoints are: (i) pharmacokinetics; and (ii) development of anti-drug antibodies (ADA) after IVT injection of the co-formulation.

[00250] The exploratory endpoints are: (1) change in BCVA from baseline; (2) change in central retinal thickness from baseline (measured by OCT) at week 12 and week 24; and (3) the number of PRN aflibercept injections from week 12 through week 20.

[00251] For AMD patients only: (i) change in CNV area from baseline (measured by FA) at week 12 and week 24; (ii) change in total lesion area from baseline (measured by FA) at week 12 and week 24; (iii) change in area of leakage from baseline (measured by FA) at week 12 and week 24.

[00252] For DME patients only: change in DRSS (measured by FP) from baseline at week 12 and week 24

Rationale for Study Design

[00253] Anti-VEGF therapy (*e.g.*, aflibercept) is standard of care treatment for neovascular AMD and DME. Targeting both the VEGF and Angiopoeitin-2 (Ang-2) pathways in neovascular eye disease may result in additional efficacy over treatment with an anti-VEGF therapy alone, and also has the possibility of providing a longer duration of action resulting in a longer treatment interval. In a preclinical model of retinal neovascularization and chronic vascular leak induced by DL-AAA in rabbit, treatment with intravenous (IV) mAb1 (15 mg/kg) with IVT aflibercept (125µg/50µl) resulted in a suppression of vascular leak through week 8, compared to only week 3 with aflibercept alone (see details in Example 2).

[00254] In another study, the effect of mAb1 on retinal vascular development was compared to that of aflibercept, or to treatment with both mAb1 and aflibercept. Doses of mAb1 (25 mg/kg, subcutaneous [SC]) and aflibercept (25 mg/kg, SC) utilized in this experiment were in excess of the minimal doses required to obtain maximal suppression of retinal angiogenesis when each drug is used as a single agent. Administration of either mAb1 or aflibercept on P3 significantly reduced the mean vascularized area of the retina measured at P6 by 36% and 42%, respectively, relative to hFc controls. Moreover, the mean vascularized area of the retina was significantly smaller in the

animals treated with both mAb1 and aflibercept, compared to animals treated with either agent alone, being reduced by 68%, representing a near complete arrest of retinal vascular development over the treatment period (see details in Example 3).

Rationale for Dose Selection

[00255] The starting dose is composed of a co-formulation of 0.5 mg mAb1: 2 mg aflibercept, administered via IVT injection in the study eye. The 2 mg dose of aflibercept is equivalent to that presently approved and marketed for the treatment of wet AMD and central retinal vein occlusion.

[00256] The 0.5 mg: 2 mg dose of the co-formulation is one-half of the lowest dose administered IVT bilaterally to cynomolgus monkeys during the Good Laboratory Practice toxicology study. Doses of up to 6 mg mAb1 in combination with 2 mg aflibercept, and 6 mg mAb1 alone were well-tolerated in the monkeys.

[00257] The rationale for this starting dose from a biologic standpoint is based on the DL-AAA model for neovascularization in the rabbit eye. In that model, a co-formulation of IVT aflibercept (0.125 mg) and mAb1 (0.5 mg) significantly increased the duration of anti-leak effects from 3 weeks to 8 weeks compared to aflibercept monotherapy. Extrapolating to the human eye (which is approximately 4 times larger than the rabbit eye) doses ranging from 0.5 mg to 0.6 mg would be expected to show biologic activity. Thus an initial dose of 0.5 mg is reasonable from a pharmacologic standpoint (see Example 2).

[00258] Safety of the proposed initial dose is supported by a 13-week IVT toxicology study in monkeys which utilized doses of mAb1 of 1, 3, and 6 mg in combination with 2 mg aflibercept bilaterally and included the 6 mg dose of mAb1 alone. Given that the volume of the human vitreous cavity is approximately 4 times that of the monkey vitreous cavity, the initial starting dose is effectively 8 times lower than the lowest dose in the monkey study, and approximately 48 times lower than the highest planned dose. Thus, the lowest dose used in the monkey IVT study provides an adequate safety margin for the proposed clinical starting dose.

Study Design

[00259] The study consists of a screening period (day -21 to day -1), a baseline visit (day 1), a treatment period (day 1 through day 57), follow-up (day 85 through day 141) and an end of study visit [day 169 (week 24)]. On day 1/baseline, eligible patients undergo safety assessments prior to receiving the first dose of study drug.

[00260] On day 1, day 29 and day 57, patients receive an injection of the co-formulation (mAb1 and aflibercept) or mAb1 alone for a total of 3 doses. The initial cohort receive the co-formulation at 0.5 mg: 2 mg. Planned administration of the co-formulation and mAb1 alone is outlined in Table 43.

If a maximum tolerated dose (MTD) is determined, the dose of mAb1 given alone will correspond to the highest dose of mAb1 administered within the co-formulation of mAb1 and aflibercept.

Table 43: Planned Dose Levels

Cohort (n=4 – 8)	IVT mAb1:aflibercept	IVT mAb1
1	0.5 mg : 2 mg	
2	1 mg : 2 mg	
3	3 mg : 2 mg	
4	6 mg : 2 mg	
5		6 mg

[00261] The last dose of study drug is administered at week 8. Intravitreal aflibercept injection (2 mg) is available to all patients beginning at week 12 for the study eye through week 20. Patients receiving aflibercept at week 12 will continue to receive aflibercept treatment with the possibility of up to monthly injections.

[00262] Patients are evaluated at study visits for ocular and systemic safety (including ophthalmic exams, laboratory assessments, etc.) and efficacy (optical coherence tomography [OCT], fluorescein angiography [FA]/fundus photography [FP], fundus autofluorescence [FAF] and BCVA using the 4-meter Early Treatment Diabetic Retinopathy Study [ETDRS] protocol), and are followed to week 24 (end of study).

Study Eye/Fellow Eye Selection: Only 1 eye per patient is enrolled in the study. The enrolled eye will be designated as the study eye. The other eye is considered to be the fellow eye.

[00263] For patients who meet eligibility criteria in both eyes, the eye with the worse BCVA score is selected as the study eye. If a patient has similar BCVA scores in both eyes, the eye with the clearest media is selected as the study eye. If the ocular media of both eyes are similar in clarity, the patient's non-dominant eye (if identifiable) is selected as the study eye. If neither eye is dominant, the right eye is designated as the study eye.

Study Cohorts

[00264] Approximately 20 to 40 patients are enrolled in this dose escalation study. Four sequential ascending dose cohorts are planned for the co-formulated mAb1 and aflibercept (0.5 mg : 2 mg, 1 mg : 2 mg, 3 mg : 2 mg, and 6 mg : 2 mg) and 1 cohort is planned for mAb1 alone (6 mg). Each dose cohort initially consists of 4 patients, 2 DME and 2 AMD. If 1 dose limiting toxicity (DLT) occurs, the cohort can be expanded to include 2 additional DME patients and/or 2 additional AMD patients. Cohorts 4 and 5 can be run in parallel if cohort 4 is reached.

Dose Escalation and Study Stopping Rules

[00265] The decision to escalate to the next higher dose level of co-formulated mAb1 and aflibercept is based upon safety and tolerability information from both AMD and DME patients evaluated during the ongoing cohort and reviewed. The decision to escalate can take place after the last patient in a cohort (both AMD and DME) is observed for at least 1 week after receiving their first dose of study medication. The mAb1 alone cohort will be enrolled either at the MTD or the highest dose (6 mg), if an MTD is not identified. If an MTD is not identified, enrollment in this cohort will be concurrent with the 6 mg : 2 mg mAb1: aflibercept co-formulation cohort.

[00266] If a DLT is observed, expansion of the cohort may be considered. Expansion of a cohort may involve 2 additional AMD patients and/or 2 additional DME patients. The rationale to increase the number of patients in each cohort beyond 4 following a DLT event is to enhance the ability to differentiate certain AEs that are known to occur sporadically with IVT injections (*e.g.*, hypersensitivity responses or moderate to severe intraocular inflammation) from a true DLT event related to study drug exposure. Cohort expansion will not be automatic. Instead, the findings will be discussed to adjudicate the clinical significance of any potential DLT to determine if cohort expansion or dose escalation to the next higher dose level of the co-formulation will occur.

[00267] All patients are observed for at least 7 days after receiving co-formulated mAb1 and aflibercept before opening enrollment in the next dose cohort (although screening for the next dose cohort may begin prior to confirmation that the current dose is safe). Escalation to the next dose cohort will occur once all of the initial 4 patients enrolled in a cohort have completed day 8 (visit 7) safety assessments and the data have been reviewed. Please refer to Table 44 for the dose escalation and stopping rules.

Table 44: Dose Escalation and Stopping Rules

No.of Patients with DLT(s) per Cohort	**Action**
0 out of 4	Escalate to the next dose level
1 out of 4 patients with AMD or DME	Consider expanding the current dose level by 2 AMD patients and/or 2 DME patients up to a total of 8 patients
1 out of 6 or 8	Escalate to the next dose level
≥2 in any cohort	Stop enrollment at the current dose level.

[00268] If 2 or more DLTs are observed, dosing will be suspended until a safety review has been conducted. The outcome of the safety review will be a decision to: continue the study as planned, expand the current dose cohort, or stop dosing at the current dose. In this case, the observed toxicity will be considered to be dose-limiting, and the dose below the highest dose administered will be considered the MTD. If 1 grade 4 AE or grade 4 serious adverse event (SAE) is observed in any cohort (original dosing cohort or expansion cohort), dosing will be suspended and a comprehensive safety review will be conducted prior to consideration of further dosing.

Dose-Limiting Toxicities

[00269] A DLT is defined as the following: A grade 2 or 3 ocular toxicity as determined by the Ocular Toxicity Grading Scale or a grade 3 or 4 toxicity in the Food and Drug Administration (FDA) September 2007 Guidance for Industry, Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

Maximum Tolerated Dose

[00270] The MTD is defined as the dose level immediately below the level at which dosing is stopped due to the occurrence of 2 or more DLTs. If the study is not stopped due to the occurrence of a DLT, it will be considered that the MTD has not been determined.

Study Population

[00271] The target population are men and women 50 years and older with neovascular AMD, or men and women 18 years and older with clinically significant DME with central involvement.

[00272] Inclusion criteria: A patient must meet the following criteria to be eligible for inclusion in the study: (1) For patients with AMD: a. active subfoveal choroidal neovascularization (CNV) secondary to AMD, including juxtafoveal lesions that affect the fovea as evidenced by FA or OCT in the study eye, as determined by the investigator; and b. Men or women ≥50 years and older. For patients with DME: c. Patients with clinically significant DME with central involvement (≥300 µm in the central subfield on spectral domain OCT); and d. Men or women ≥18 years and older. (2) Willing and able to comply with clinic visits and study-related procedures; and (3) Provide signed informed consent.

[00273] Exclusion criteria: A patient who meets any of the following criteria is excluded from the study: (1) (a) For patients with neovascular AMD: Evidence of CNV due to any cause other than AMD in either eye; Evidence of DR or DME in either eye; (b) For patients with DME: Evidence of neovascular AMD or CNV due to any cause in either eye; (2) Prior aflibercept in either eye; (3) IVT bevacizumab, ranibizumab, or pegaptanib sodium in the study eye within 8 weeks of day 1 or an AE with any of these previous treatments that would preclude administration of drug in this study; (4) Any prior treatment with angiopoietin inhibitors; (5) Any prior systemic (IV) anti-VEGF administration; (6) History of vitreoretinal surgery in the study eye; (7) Pan retinal laser photocoagulation or macular laser photocoagulation in the study eye within 3 months of the screening visit; (8) Previous use of intraocular or periocular corticosteroids in the study eye within 4 months of screening; (9) Intraocular pressure (IOP) ≥25 mm Hg in the study eye at screening/baseline; (10) Evidence of infectious blepharitis, keratitis, scleritis, or conjunctivitis in either eye at screening/baseline; (11) Any intraocular inflammation/infection in either eye within 3 months of the screening visit; (12) Current iris neovascularization, vitreous hemorrhage, or tractional retinal detachment visible at the screening visit in the study eye; (13) Inability to obtain photographs, FA, or OCT to document CNV, eg, due to media opacity, allergy to fluorescein dye or

lack of venous access at screening/baseline; (14) Uncontrolled diabetes mellitus, in the opinion of the investigator; (15) Uncontrolled blood pressure (defined as systolic >160 mm Hg or diastolic >95 mm Hg while patient is sitting); (16) History of cerebrovascular accident or myocardial infarction within 180 days of day 1; (17) Renal failure, dialysis, or history of renal transplant; (18) Known sensitivity to doxycycline or similar compound (ie, tetracyclines); (19) Known sensitivity to any of the compounds of the study formulation; (20) Pregnant or breastfeeding women; and (210 Sexually active men or women of childbearing potential who are unwilling to practice adequate contraception during the study (adequate contraceptive measures include stable use of oral contraceptives or other prescription pharmaceutical contraceptives for 2 or more menstrual cycles prior to screening; intrauterine device; bilateral tubal ligation; vasectomy; condom plus contraceptive sponge, foam, or jelly, or diaphragm plus contraceptive sponge, foam, or jelly).

Study Treatments

Investigational and Reference Treatments

[00274] Co-formulated mAb1 and aflibercept is a drug product that is composed of mAb1 (anti-Ang2 antibody) and aflibercept. It will be supplied for this study as an aqueous solution in sterile, single-use 2 mL glass vials for IVT administration, in the following concentrations (with progressively higher concentrations to be used for the progressively higher doses in dose escalation): 10 : 40 mg/mL, 20 : 40 mg/mL; 60 : 40 mg/mL; and 120 : 40 mg/mL (mAb1: aflibercept).

[00275] The mAb1 alone drug product is also supplied for this study as an aqueous solution in sterile, single-use 2 mL glass vials for IVT administration, at a concentration of 120 mg/mL.

[00276] The co-formulation and mAb1 is delivered via IVT injection and the injection volume will be 50 µl (0.05 cc). There will be a 0.050 ml minimum withdrawable content. Each vial contains a withdrawable volume of 0.3 mL of mAb1.

[00277] Patients will be given 3 doses of study drug. Study drug is administered on day 1, day 29 and day 57 by the investigator, or other qualified study personnel. Patients are enrolled in order to receive one of the following co-formulation or mAb1 treatment regimens:

cohort 1: 0.5 mg : 2 mg (mAb1: aflibercept)
cohort 2: 1 mg : 2 mg (mAb1: aflibercept)
cohort 3: 3 mg : 2 mg (mAb1: aflibercept)
cohort 4: 6 mg : 2 mg (mAb1: aflibercept)
cohort 5: 6 mg (mAb1 alone)

Background Treatments

[00278] Study Eye Treatment: Intravitreal aflibercept injections are supplied in sterile, sealed vials with a volume sufficient to prepare a syringe with 50 uL at a concentration of 40 mg/mL. Beginning

at week 12 (and continuing through week 20), patients will be eligible to receive monthly aflibercept treatment (2 mg) in the study eye, if any of the re-treatment criteria listed below are satisfied: (1) There is a >50 µm increase in central retinal thickness on OCT compared to the lowest previous measurement. (2) There are new or persistent cystic retinal changes or subretinal fluid on OCT, or persistent diffuse edema in the central subfield on OCT. (3) A loss of 5 or more letters from the best previous measurement in conjunction with any increase in retinal thickness in the central subfield on OCT. (4) An improvement of BCVA between the current and most recent visit of ≥5 letters.

[00279] Fellow Eye Treatment: At week 4, aflibercept (2 mg) will be made available to patients with AMD or DME in the fellow eye at screening, or who are diagnosed with AMD or DME during the trial. The fellow eye will be assessed for safety during the trial, but will not be considered a study eye. The patient's fellow eye may, at the discretion of the investigator, receive treatment on the same day as the treatment of the study eye. All fellow eye treatments must be recorded on the electronic case report form (CRF) as a procedure for the fellow eye.

[00280] Patients who receive treatment for the fellow eye will not be required to be withdrawn from the study. Study assessments and all AEs for the fellow eye will be collected.

Prohibited Medications

[00281] Study Eye: Patients may not receive any standard or investigational agents for AMD or DME treatment in the study eye other than their assigned study treatment with IVT co-formulation or IVT mAb1, and, if needed, aflibercept, as specified in this protocol. This includes medications administered locally (eg, IVT, topical, juxtascleral or periorbital routes), as well as those administered systemically, with the intent of treating neovascular AMD or DME in the study eye.

[00282] Fellow Eye: Starting at week 4, if the fellow eye has DME involving or threatening the center of the macula or neo-vascular AMD, aflibercept (2 mg) may be administered. Other conditions in the fellow eye may be treated with approved therapies; however they must be administered locally.

[00283] Non-ocular Systemic: Non-ocular (systemic) standard or investigational treatments for neo-vascular AMD and DME of the study or fellow eye are not permitted. Systemic anti-angiogenic agents will not be permitted during the study.

Study Procedures

[00284] Safety and tolerability is assessed by monitoring/evaluation of treatment-emergent adverse events (TEAEs), physical examinations, vital signs, electrocardiograms (ECGs), and clinical evaluations (hematology, blood chemistry and urinalysis). Ocular safety is assessed by ophthalmic examinations (slit lamp, indirect ophthalmoscopy, intraocular pressure [IOP], spectral domain OCT, BCVA, FAF and information from FP and FA. Serum samples will be collected for assessing mAb1

and plasma PK samples for aflibercept. Serum samples to assess ADA responses will be collected.

[00285] Best Corrected Visual Acuity (BCVA): Visual function of the study eye and the fellow eye is assessed using the 4M ETDRS protocol (The Early Treatment Diabetic Retinopathy Study Group 1985) at 4 meters at each study visit at screening, baseline, and week 1, 4, 6, 8, 12, 16, 20 and 24 after treatment.

[00286] Fluorescein Angiography/Fundus Photography (FA/FP): The anatomical state of the retinal vasculature of the study eye and the fellow eye is evaluated by funduscopic examination, FA, and FP at time points each study visit at screening, baseline, and week 1, 4, 6, 8, 12, 16, 20 and 24 after treatment. At a minimum, information on the following variables will be collected:

For AMD only: total lesion area, CNV area, classic CNV area, and area of fluorescein leakage.

For DME only: Diabetic Retinopathy Severity Score (DRSS).

[00287] Certified photographers will perform FA and FP in both eyes at the time points listed above. Fundus and angiographic images are sent to the independent reading center. The study eye will be the transit eye. All FA and FP will be archived at the site as part of the source documentation.

[00288] Spectral Domain Optical Coherence Tomography: Retinal and lesion characteristics are evaluated using spectral domain OCT at time points each study visit at screening, baseline, and week 1, 4, 6, 8, 12, 16, 20 and 24 after treatment.

[00289] Images are captured and transmitted at the study site by OCT technicians using spectral domain OCT for the study eye and fellow eye. Optical coherence tomography images are sent to the independent reading center where images for the study eye will be read. All OCTs will be electronically archived at the study sites as part of the source documentation. Optical coherence tomography technicians will be certified by the reading center to ensure consistency and quality in image acquisition and will be masked to patients' dose level of the co-formulation.

[00290] Fundus Autofluorescence: Anatomic characteristics of the retina are also evaluated using autofluorescence. Certified photographers will perform FAF at time points each study visit at screening, baseline, and week 1, 4, 6, 8, 12, 16, 20 and 24 after treatment. Images will be sent to the independent reading center. All images will be archived at the site as part of the source documentation.

[00291] Intraocular pressure: Intraocular pressure of the study eye is measured at each study visit, using Goldmann applanation tonometry or Tono-pen™. The same method of IOP measurement must be used in each patient throughout the study. On visits where an aflibercept treatment is given, IOP must be measured pre-treatment (bilateral) and at approximately 30 minutes post-treatment (study eye only).

[00292] Slit Lamp Examination: The anterior segment and the anterior vitreous is examined using a slit lamp at time points each study visit at screening, baseline, and week 1, 4, 6, 8, 12, 16, 20 and 24 after treatment. Both eyes are examined. Examination of the fundus is also conducted by indirect ophthalmoscopy.

[00293] Anterior chamber flare and cells are graded. The intensity of the cellular reaction in the anterior chamber is graded according to the number of inflammatory cells seen in a 1 x 3-mm high-powered beam at full intensity at a 45° to 60° angle. In addition, vitreal inflammatory response is graded.

[00294] Indirect Ophthalmoscopy: Indirect ophthalmoscopy is performed at time points each study visit at screening, baseline, and week 1, 4, 6, 8, 12, 16, 20 and 24 after treatment. It will be performed bilaterally predose, and in the study eye immediately after administration of study drug on days when study drug is administered. The appropriate lenses and optics are used, ie, a head mounted or handheld ophthalmoscope for the periphery, and a slit lamp for the central fundus. The pupil is dilated, and examination of the fundus is done on both eyes.

[00295] Safety non-ocular: Vital signs, including temperature, sitting blood pressure, pulse, and respiration is collected, a complete and thorough physical examination, a standard 12-lead ECG as well as standard laboratory testing for hematology, chemistry, urinalysis, and pregnancy testing will be carried out predose at time points each study visit at screening, baseline, and week 1, 4, 6, 8, 12, 16, 20 and 24 after treatment.

Safety

[00296] Safety and tolerability is assessed by monitoring/evaluation of treatment-emergent adverse events (TEAEs), physical examinations, vital signs, electrocardiograms (ECGs), and clinical evaluations (hematology, blood chemistry and urinalysis).

[00297] An adverse event (AE) is any untoward medical occurrence in a patient administered a study drug which may or may not have a causal relationship with the study drug. Therefore, an AE is any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease which is temporally associated with the use of a study drug, whether or not considered related to the study drug.

[00298] A serious adverse event (SAE) is any untoward medical occurrence that at any dose: results in death, is life-threatening, requires in-patient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, and/or is an important medical event. Criteria for serious sight-threatening ocular AEs include the following: (1) AE causes a decrease in BCVA of >30 letters (compared with the most recent assessment of BCVA). (2) AE causes a decrease in VA to the level of light perception or worse. (3) AE requires surgical intervention (*e.g.*, vitreous tap or biopsy with IVT injection of

anti-infectives, laser or retinal cryopexy with gas) to prevent permanent loss of sight. (4) AE is associated with severe intraocular inflammation (ie, 4 + anterior chamber cell/flare or 4 + vitritis). (5) In the opinion of the investigator, AE may require medical intervention to prevent permanent loss of sight.

Results

[00299] Preliminary analysis of safety results indicated that the co-formulation was well tolerated in both AMD and DME patients with a favorable safety profile.

[00300] Tables 45 and 46 show the baseline demographics of patients with AMD and DME respectively.

Table 45: Baseline demographics in AMD patients

	Cohort 1 0.5mg:2mg (N=2)	Cohort 2 1mg:2mg (N=2)	Cohort 3 3mg:2mg (N=2)	Cohort 4 6mg:2mg (N=2)	Cohort 5 6mg mAb1 (N=4)	Overall (N=10)
Age						
Years	82	71	72	78	76	76
Min:Max	78 : 86	61 : 81	57 : 87	78 : 79	64 : 89	57 : 89
Gender, n (%)						
Female	1	1	1	0	1	4 (40%)
Male	1	1	1	2	1	6 (60%)
Race, n (%)						
White	2	2	2	2	2	10 (100%)
Black	0	0	0	0	0	0
Asian	0	0	0	0	0	0

Table 46: Baseline demographics in DME patients

	Cohort 1 0.5mg:2mg (N=2)	Cohort 2 1mg:2mg (N=2)	Cohort 3 3mg:2mg (N=2)	Cohort 4 6mg:2mg (N=2)	Cohort 5 6mg REGN910 (N=2)	Overall (N=10)
Age						
years	66	55	66	61	57	61
Min:Max	56:77	54:57	65:68	55:67	57:58	54 : 77
Gender, n (%)						
Female	1	2	2	1	1	7 (70%)
Male	1	0	0	1	1	3 (30%)
Race, n (%)						

White	2	0	2	1	2	7 (70%)
Black	0	1	0	1	0	2 (20%)
Asian	0	1	0	0	0	1 (10%)

[00301] The baseline characteristics of AMD and DME patients are summarized in Tables 47 and 48, respectively.

Table 47: Baseline characteristics of AMD patients

N (All Cohorts)	10
BCVA	61.7 (12.41)
Min:Max	35 : 76
IOP	17.0 (3.40)
Min:Max	10 : 22
CRT(μm)	415.6 (105.69)
Min:Max	263 : 621

Table 48: Baseline characteristics of DME patients

N (All Cohorts)	10
BCVA	59.7 (11.92)
Min:Max	31 : 75
IOP	14.9 (3.14)
Min:Max	9 : 20
CRT(μm)	453.6 (129.87)
Min:Max	310 : 689

[00302] Results to date (at 12 weeks) demonstrate improvement in vision (increase in visual acuity of up to 20 letters) and retinal morphology (decrease in central subfield thickness) at all dose levels. Duration of effect was extended at higher doses (for the 3mg:2mg and 6mg:2mg doses).

[00303] At 24 weeks after treatment, it is expected that the visual acuity gains will be maintained or improved in patients with AMD or DME.

[00304] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. A method for treating a vascular eye disease or disorder comprising intravitreally administering a therapeutically effective amount of a pharmaceutical composition comprising an angiopoietin-2 (Ang-2) inhibitor to a subject in need thereof.

2. The method of claim 1, wherein the pharmaceutical composition further comprises a vascular endothelial growth factor (VEGF) antagonist.

3. The method of any one of claims 1 – 2, wherein the pharmaceutical composition comprises about 10 – 120 mg/mL of the Ang-2 inhibitor.

4. The method of claim 3, wherein the pharmaceutical composition comprises 10 mg/mL, 20 mg/mL, 60 mg/mL or 120 mg/mL of the Ang-2 inhibitor.

5. The method of any one of claims 2 – 4, wherein the pharmaceutical composition comprises about 40 mg/mL of the VEGF antagonist.

6. The method of any one of claims 1 – 5, wherein the Ang-2 inhibitor is administered at a dose of from 0.5 mg to 10 mg to the subject in need thereof.

7. The method of any one of claims 2 – 6, wherein the VEGF antagonist is administered at a dose of from 50 µg to 5 mg to the subject in need thereof.

8. The method of any one of claims 1 – 7, wherein the eye disease or disorder is selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal neovascularization, central retinal vein occlusion, branched retinal vein occlusion, polypoidal choroidal vasculopathy, and choroidal neovascularization.

9. The method of any one of claims 1 – 8, wherein the eye disease or disorder is age-related macular degeneration.

10. The method of any one of claims 1 – 8, wherein the eye disease or disorder is diabetic macular edema.

11. A method for inhibiting retinal angiogenesis comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor in combination with a VEGF antagonist to a subject in need thereof.

12. The method of claim 11, wherein the Ang-2 inhibitor is intravitreally administered in combination with the VEGF antagonist.

13. The method of any one of claims 11 – 12, wherein the Ang-2 inhibitor is administered at a dose of about 1 – 10 µg.

14. The method of any one of claims 11 – 13, wherein the VEGF antagonist is administered at a dose of about 1 – 10 µg.

15. The method of claim 11, wherein the Ang-2 inhibitor is subcutaneously administered in combination with the VEGF antagonist.

16. The method of claim 15, wherein the Ang-2 inhibitor is administered at a dose of 5 – 30 mg/kg of the subject's body weight.

17. The method of claim 15 or 16, wherein the VEGF antagonist is administered at a dose of 5 – 30 mg/kg of the subject's body weight.

18. The method of any one of claims 11 – 17, wherein the combined treatment reduces vascular area by at least 65% as compared to administration of either Ang-2 inhibitor or VEGF antagonist alone.

19. The method of any one of claims 11 -18, wherein the retinal angiogenesis is associated with an eye disease or disorder selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal neovascularization, central retinal vein occlusion, branched retinal vein occlusion, polypoidal choroidal vasculopathy, and choroidal neovascularization.

20. A method of inhibiting retinal neovascularization comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor in combination with a VEGF antagonist to a subject in need thereof.

21. The method of claim 20, wherein the Ang-2 inhibitor is intravitreally administered in combination with the VEGF antagonist.

22. The method of claim 21, wherein the Ang-2 inhibitor is administered at a dose of from 0.1 to 10.0 mg and the VEGF antagonist is administered at a dose of about 0.1 mg to 5 mg.

23. The method of claim 20, wherein the Ang-2 inhibitor is intravenously administered once every 2 weeks at a dose of 15 mg/kg of the subject's body weight.

24. The method of claim 23, wherein the VEGF antagonist is intravitreally administered at a dose of from 0.1 to 5 mg.

25. The method of any one of claims 20 – 24, wherein the retinal neovascularization is associated with an eye disease or disorder selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, central retinal vein occlusion and branched retinal vein occlusion.

26. A method for inhibiting vascular leak comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor in combination with a VEGF antagonist to a subject in need thereof.

27. The method of claim 26, wherein the vascular leak is inhibited for at least 3 weeks as compared to a subject who has been administered the VEGF antagonist alone.

28. The method of claim 27, wherein the vascular leak is inhibited for more than 3 weeks as compared to a subject who has been administered the VEGF antagonist alone.

29. The method of claim 26 or 28, wherein the vascular leak is inhibited for at least 8 weeks as compared to a subject who has been administered the VEGF antagonist alone.

30. The method of any one of claims 26 – 29, wherein the vascular leak is inhibited for at least 10 weeks as compared to a subject who has been administered the VEGF antagonist alone.

31. The method of any one of claims 26 – 30, wherein the Ang-2 inhibitor is administered at a dose of about 5 – 50 mg/kg of the subject's body weight in combination with 0.05 – 2 mg of the VEGF antagonist.

32. The method of claim 31, wherein the Ang-2 inhibitor is administered at a dose of about 15 mg/kg of the subject's body weight in combination with 125 µg of the VEGF antagonist.

33. The method of any one of claims 26 – 30, wherein the Ang-2 inhibitor is administered intravenously or subcutaneously.

34. The method of any one of claims 26 – 33, wherein the VEGF antagonist is administered intravitreally.

35. The method of any one of claims 26 – 30, wherein the Ang-2 inhibitor is administered intravitreally in combination with the VEGF antagonist.

36. The method of claim 35, wherein the Ang-2 inhibitor is administered at a dose of about 500 µg in combination with about 125 µg of the VEGF antagonist.

37. The method of any one of claims 26 – 36, wherein the vascular leak is associated with an eye disease or disorder selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal neovascularization, central retinal vein occlusion, branched retinal vein occlusion, polypoidal choroidal vasculopathy, and choroidal neovascularization.

38. The method of any one of claims 26 – 37, wherein the vascular leak is associated with retinal neovascularization.

39. A method for suppressing vascular leak in a subject with an eye disease, the method comprising administering a single dose of a VEGF antagonist followed by one or more doses of a pharmaceutical composition comprising an Ang-2 inhibitor to the subject in need thereof.

40. The method of claim 39, wherein the VEGF antagonist is administered at a dose of from 100 to 500 µg.

41. The method of any one of claims 39 or 40, wherein the VEGF antagonist is intravitreally administered.

42. The method of any one of claims 39 – 41, wherein the Ang-2 inhibitor is intravenously or subcutaneously administered.

43. The method of any one of claims 39 – 42, wherein the one or more doses of the Ang-2 inhibitor are administered once every 2 weeks.

44. The method of any one of claims 39 – 43, wherein the one or more doses of the Ang-2 inhibitor each comprise 15 mg/kg of the subject's body weight.

45. The method of any one of claims 39 – 44, wherein the vascular leak is inhibited for at least 4 weeks as compared to a subject who has been administered the VEGF antagonist alone.

46. The method of claim 45, wherein the vascular leak is inhibited for more than 4 weeks as compared to a subject who has been administered the VEGF antagonist alone.

47. The method of claim 45, wherein the vascular leak is inhibited for at least 8 weeks as compared to a subject who has been administered the VEGF antagonist alone.

48. The method of claim 47, wherein the vascular leak is inhibited for at least 10 weeks as compared to a subject who has been administered the VEGF antagonist alone.

49. The method of any one of claims 39 – 48, wherein the vascular leak is associated with an eye disease or disorder selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal neovascularization, central retinal vein occlusion, branched retinal vein occlusion, polypoidal choroidal vasculopathy, and choroidal neovascularization.

50. The method of any one of claims 39 – 49, wherein the vascular leak is associated with retinal neovascularization.

51. A method of reducing the number of intravitreal injections administered to a subject with an eye disease, the method comprising sequentially administering a first initial dose following by one of more secondary doses of a pharmaceutical composition comprising a therapeutically effective amount of an Ang-2 inhibitor in combination with a VEGF antagonist to the subject in need thereof, wherein the intravitreal administration of the VEGF antagonist is reduced to once every 9 weeks as compared to a subject who has been administered the VEGF antagonist alone.

52. The method of claim 51, wherein the one or more secondary doses comprises 2 – 10 doses of the pharmaceutical composition.

53. The method of claim 52, wherein at least 2 secondary doses of the pharmaceutical composition are administered to the subject, and wherein each secondary dose is administered 1 to 4 weeks after the immediately preceding dose.

54. The method of any one of claims 51 – 53, wherein the initial dose and the one or more secondary doses of the pharmaceutical composition each comprise about 0.5 – 10 mg of the Ang-2 inhibitor.

55. The method of any one of claims 51 – 54, wherein the Ang-2 inhibitor is administered intravenously or subcutaneously.

56. The method of any one of claims 51 – 55, wherein the VEGF antagonist is administered at a dose of about 1 – 5 mg.

57. The method of any one of claims 51 – 56, wherein the eye disease is selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal neovascularization, and choroidal neovascularization.

58. A method for inhibiting choroidal neovascularization comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an Ang-2 inhibitor to a subject in need thereof.

59. The method of claim 58, wherein the Ang-2 inhibitor is subcutaneously administered at a dose of about 10 – 50 mg/kg of the subject's body weight.

60. The method of claims 59, wherein the Ang-2 inhibitor is administered at a dose of 25 mg/kg of the subject's body weight.

61. The method of any one of claims 58 – 60, wherein the choroidal neovascularization is associated with an eye disease or disorder selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, central retinal vein occlusion, branched retinal vein occlusion, and polypoidal choroidal vasculopathy.

62. The method of any one of claims 1 – 61, wherein the Ang-2 inhibitor is an anti-Ang-2 antibody or antigen binding fragment thereof.

63. The method of claim 62, wherein the antibody or antigen binding fragment comprises the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO: 1 and the CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO: 2.

64. The method of claim 63, wherein the antibody or antigen binding fragment comprises a heavy chain CDR1 (HCDR1) having the amino acid sequence of SEQ ID NO: 3, a HCDR2 having the amino acid sequence of SEQ ID NO: 4, a HCDR3 having the amino acid sequence of SEQ ID NO: 5, a light chain CDR1 (LCDR1) having the amino acid sequence of SEQ ID NO: 6, a LCDR2 having the amino acid sequence of SEQ ID NO: 7, and a LCDR3 having the amino acid sequence of SEQ ID NO: 8.

65. The method of claim 64, wherein the antibody or antigen binding fragment thereof comprises a HCVR having the amino acid sequence of SEQ ID NO: 1 and a LCVR having the amino acid sequence of SEQ ID NO: 2.

66. The method of any one of claims 2 – 57, wherein the VEGF antagonist comprises a VEGF receptor-based chimeric molecule (VEGF Trap).

67. The method of claim 66, wherein the VEGF Trap comprises one or more immunoglobulin (Ig)-like domains of VEGFR1, one or more Ig-like domains of VEGFR2, and a multimerizing domain.

68. The method of claim 67, wherein the VEGF Trap comprises Ig-like domain 2 of VEGFR1, Ig-like domain 3 of VEGFR2, and a multimerizing domain.

69. The method of claim 66, wherein the VEGF Trap is aflibercept.

70. The method of any one of claims 2 – 57, wherein the VEGF antagonist consists of a dimer of two polypeptides consisting of amino acids 27-457 of SEQ ID NO: 11.

71. A stable liquid formulation comprising: (i) a vascular endothelial growth factor (VEGF) antagonist; (ii) an antibody that binds specifically to human angiopoietin-2 (hAng-2); (iii) a buffer at pH of 6.2 ± 0.3; (iv) a non-ionic detergent; (v) a tonicity agent; and (vi) a stabilizer; wherein the VEGF antagonist consists of a dimer of two polypeptides consisting of amino acids 27-457 of SEQ ID NO: 11.

72. The formulation of claim 71, wherein the antibody comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) wherein HCDR1 has SEQ ID NO: 3, HCDR2 has SEQ ID NO: 4, HCDR3 has SEQ ID NO: 5, and three light chain CDRs (LCDR1, LCDR2 and LCDR3), wherein LCDR1 has SEQ ID NO: 6, LCDR2 has SEQ ID NO: 7, and LCDR3 has SEQ ID NO: 8.

73. The formulation of any one of claims 71 and 72, wherein the antibody comprises a heavy chain variable region (HCVR) of SEQ ID NO: 1 and a light chain variable region (LCVR) of SEQ ID NO: 2.

74. The formulation of claim 73, wherein the VEGF antagonist concentration is about 40 mg/mL ± 1.5 mg/mL.

75. The formulation of claim 74, comprising about 10 – 120 mg/mL of the antibody.

76. The formulation of claim 75, comprising the antibody at a concentration selected from the group consisting of 10 mg/mL, 20 mg/mL, 60 mg/mL, and 120 mg/mL.

77. The formulation of claim 71, comprising about 5 – 100 mg/mL of the VEGF antagonist; about 10 – 120 mg/mL of the anti-Ang-2 antibody; about 5 – 50 mM sodium phosphate, pH about 6.2; about 0.01 – 0.1% (w/v) polysorbate; about 10 – 50 mM sodium chloride; and about 1 – 20% (w/v) sucrose.

78. The formulation of claim 77, comprising the antibody at a concentration selected from the group consisting of 10 mg/ml, 20 mg/ml, 60 mg/ml, and 120 mg/ml.

79. The formulation of claim 76, wherein the buffer is sodium phosphate.

80. The formulation of claim 79, wherein the sodium phosphate concentration is 10 mM ± 1.5 mM.

81. The formulation of claim 76, wherein the non-ionic detergent is polysorbate 20.

82. The formulation of claim 81, wherein the polysorbate 20 concentration is about 0.03% w/v ± 0.0045%.

83. The formulation of claim 76, wherein the tonicity agent is sodium chloride.

84. The formulation of claim 83, wherein the sodium chloride concentration is about 40 mM ± 6.0 mM.

85. The formulation of claim 76, wherein the stabilizer is sucrose.

86. The formulation of claim 85, wherein the sucrose concentration is about 5% ± 0.75% (w/v).

87. The formulation of claim 71, wherein the VEGF antagonist concentration is 40 mg/mL ± 6.0 mg/mL, the antibody concentration is 10 mg/mL ± 1.5 mg/mL, the buffer is 10mM ± 1.5mM sodium phosphate, pH 6.2 ± 0.3, the non-ionic detergent is 0.03% w/v ± 0.0045% polysorbate 20, the tonicity agent is 40mM sodium chloride, and the stabilizer is 5% w/v ± 1.5% sucrose.

88. The formulation of claim 71, wherein the VEGF antagonist concentration is 40 mg/mL ± 6.0 mg/mL, the antibody concentration is 20 mg/mL ± 3.0 mg/mL, the buffer is 10mM ± 1.5mM sodium phosphate, pH 6.2 ± 0.3, the non-ionic detergent is 0.03% w/v ± 0.0045% polysorbate 20, the tonicity agent is 40mM sodium chloride, and the stabilizer is 5% w/v ± 1.5% sucrose.

89. The formulation of claim 71, wherein the VEGF antagonist concentration is 40 mg/mL ± 6.0 mg/mL, the antibody concentration is 60 mg/mL ± 9.0 mg/mL, the buffer is 10mM ± 1.5mM sodium phosphate, pH 6.2 ± 0.3, the non-ionic detergent is 0.03% w/v ± 0.0045% polysorbate 20, the tonicity agent is 40mM sodium chloride, and the stabilizer is 5% w/v ± 1.5% sucrose.

90. The formulation of claim 71, wherein the VEGF antagonist concentration is 40 mg/mL ± 6.0 mg/mL, the antibody concentration is 120 mg/mL ± 18.0 mg/mL, the buffer is 10mM ± 1.5mM sodium phosphate, pH 6.2 ± 0.3, the non-ionic detergent is 0.03% w/v ± 0.0045% polysorbate 20, the tonicity agent is 40mM sodium chloride, and the stabilizer is 5% w/v ± 1.5% sucrose.

91. A method for treating or ameliorating a vascular eye disease or disorder, the method comprising administering the formulation of any one of claims 71 – 90 to a subject in need thereof.

92. The method of claim 91, wherein the eye disease or disorder is selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal neovascularization, central retinal vein occlusion, branched retinal vein occlusion, polypoidal choroidal vasculopathy, and choroidal neovascularization.

93. A method for treating a vascular eye disease, the method comprising sequentially administering one or more doses of a pharmaceutical composition comprising therapeutically active amount of an anti-Ang-2 inhibitor and a VEGF antagonist to a subject in need thereof.

94. The method of claim 93, wherein the pharmaceutical composition comprises about 10 mg/mL to about 120 mg/mL of the anti-Ang-2 inhibitor.

95. The method of claim 93, wherein the pharmaceutical composition comprises about 40 mg/mL of the VEGF antagonist.

96. The method of claim 93, wherein the pharmaceutical composition comprises about 10 mg/mL to about 120 mg/mL of the anti-Ang-2 inhibitor and about 40 mg/mL of the VEGF antagonist.

97. The method of claim 93, wherein the pharmaceutical composition comprises 10 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

98. The method of claim 93, wherein the pharmaceutical composition comprises 20 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

99. The method of claim 93, wherein the pharmaceutical composition comprises 60 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

100. The method of claim 93, wherein the pharmaceutical composition comprises 120 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

101. The method of claim 93 comprising administering an initial dose of the pharmaceutical composition to the subject; followed by administering one or more secondary doses of the pharmaceutical composition to the subject, wherein each secondary dose is administered 1 to 4 weeks after the immediately preceding dose.

102. The method of claim 101, wherein at least 2 secondary doses are administered to the subject and each secondary dose is administered 4 weeks after the immediately preceding dose.

103. The method of claim 102 further comprising administering one or more tertiary doses of the pharmaceutical composition to the subject wherein each tertiary dose is administered 5 to 12 weeks after the immediately preceding dose.

104. The method of claim 103, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

105. The method of claim 93, wherein each dose of the pharmaceutical composition comprises about 0.5mg to about 10mg of the anti-Ang-2 inhibitor and about 2mg of the VEGF antagonist.

106. The method of claim 105, wherein each dose of the pharmaceutical composition comprises 10 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

107. The method of claim 105, wherein each dose of the pharmaceutical composition comprises 20 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

108. The method of claim 105, wherein each dose of the pharmaceutical composition comprises 60 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

109. The method of claim 105, wherein each dose of the pharmaceutical composition comprises 120 mg/mL of the anti-Ang-2 inhibitor and 40 mg/mL of the VEGF antagonist.

110. The method of claim 93, wherein each dose of the pharmaceutical composition is intravitreally administered to the subject.

111. The method of claim 93, wherein the vascular eye disease is selected from the group consisting of diabetic retinopathy, diabetic macular edema, age-related macular degeneration, central retinal vein occlusion, branched retinal vein occlusion, and polypoidal choroidal vasculopathy.

112. The method of claim 111, wherein the vascular eye disease is age-related macular degeneration.

113. The method of claim 111, wherein the vascular eye disease is diabetic macular edema.

114. The method of claim 93, wherein the Ang-2 inhibitor is an anti-Ang-2 antibody or antigen binding fragment thereof.

115. The method of claim 114, wherein the antibody or antigen binding fragment comprises the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO: 1 and the CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO: 2.

116. The method of claim 114, wherein the antibody or antigen binding fragment comprises a heavy chain CDR1 (HCDR1) having the amino acid sequence of SEQ ID NO: 3, a HCDR2 having the amino acid sequence of SEQ ID NO: 4, a HCDR3 having the amino acid sequence of SEQ ID NO: 5, a light chain CDR1 (LCDR1) having the amino acid sequence of SEQ ID NO: 6, a LCDR2 having the amino acid sequence of SEQ ID NO: 7, and a LCDR3 having the amino acid sequence of SEQ ID NO: 8.

117. The method of claim 116, wherein the antibody or antigen binding fragment thereof comprises a HCVR having the amino acid sequence of SEQ ID NO: 1 and a LCVR having the amino acid sequence of SEQ ID NO: 2.

118. The method of claim 93, wherein the VEGF antagonist comprises a VEGF receptor-based chimeric molecule (VEGF Trap).

119. The method of claim 118, wherein the VEGF Trap comprises one or more immunoglobulin (Ig)-like domains of VEGFR1, one or more Ig-like domains of VEGFR2, and a multimerizing domain.

120. The method of claim 119, wherein the VEGF Trap comprises Ig-like domain 2 of VEGFR1, Ig-like domain 3 of VEGFR2, and a multimerizing domain.

121. The method of claim 120, wherein the VEGF Trap is aflibercept.

122. The method of claim 93, wherein the VEGF antagonist consists of a dimer of two polypeptides consisting of amino acids 27-457 of SEQ ID NO: 11.

ABSTRACT

The present invention provides methods for treating, preventing or reducing the severity of an eye disease. The methods of the present invention comprise administering to a subject in need thereof a therapeutic composition comprising an angiopoietin-2 (Ang-2) inhibitor such as an anti-Ang-2 antibody in combination with a vascular endothelial growth factor (VEGF) antagonist (*e.g.*, aflibercept).

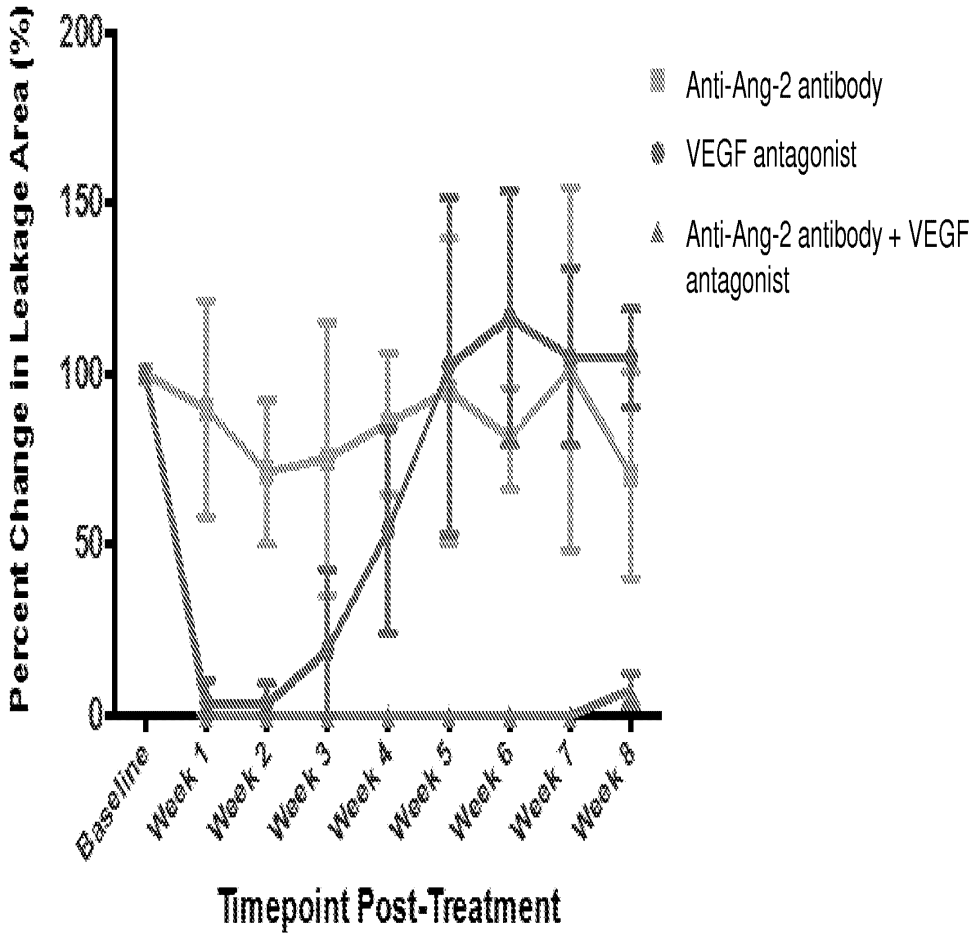

Percent Change in Leakage Area (%)
200
150
100
50
0
Baseline
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Timepoint Post-Treatment
Anti-Ang-2 antibody
VEGF antagonist
Anti-Ang-2 antibody + VEGF antagonist

Figure 1

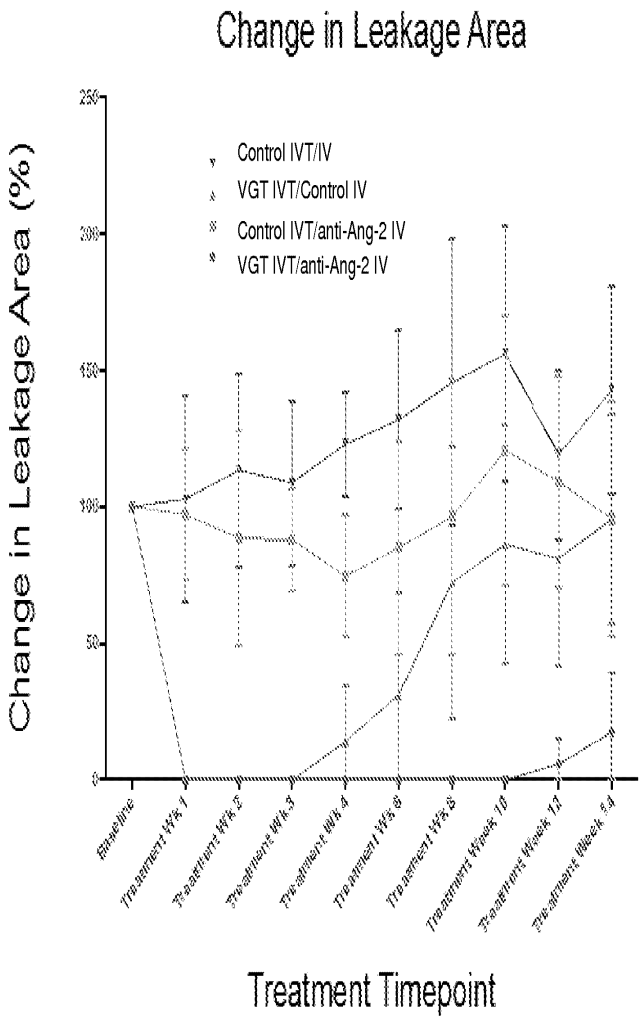

Change in Leakage Area
Change in Leakage Area (%)
Control IVT/IV
VGT IVT/Control IV
Control IVT/anti-Ang-2 IV
VGT IVT/anti-Ang-2 IV
0
50
100
150
200
250
Baseline
Treatment Wk 1
Treatment Wk 2
Treatment Wk 3
Treatment Wk 4
Treatment Wk 6
Treatment Wk 8
Treatment Week 10
Treatment Week 12
Treatment Week 14
Treatment Timepoint

Figure 2

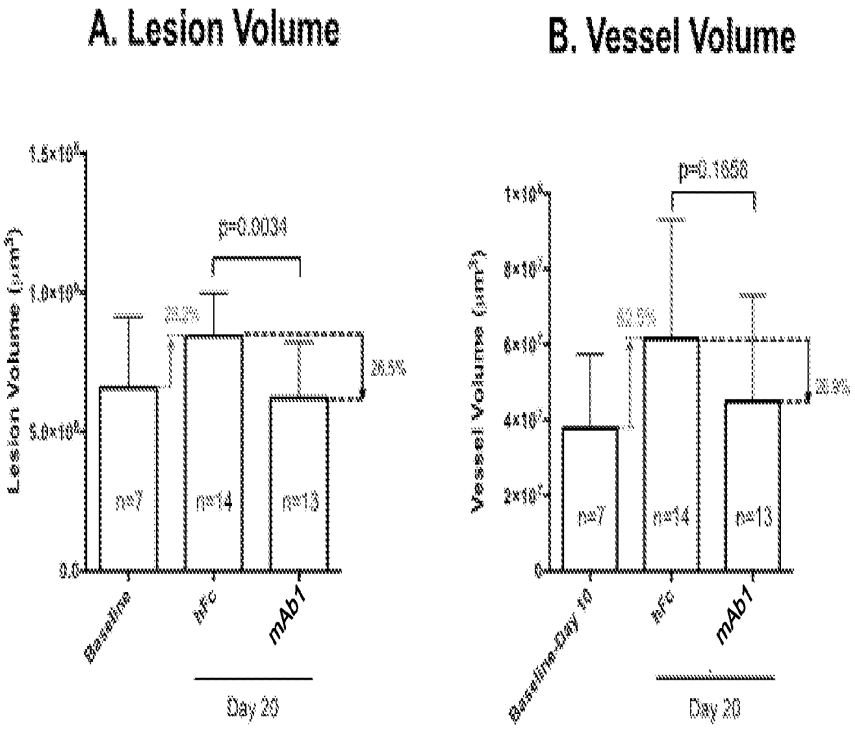

A. Lesion Volume
B. Vessel Volume
Lesion Volume (µm³)
Vessel Volume (µm³)
p=0.0034
p=0.1858
n=7
n=14
n=13
Baseline
Baseline-Day 10
hFc
mAb1
Day 28
Day 20

Figure 3

C. Vessel Density
(Vessel Volume/Lesion Volume)%

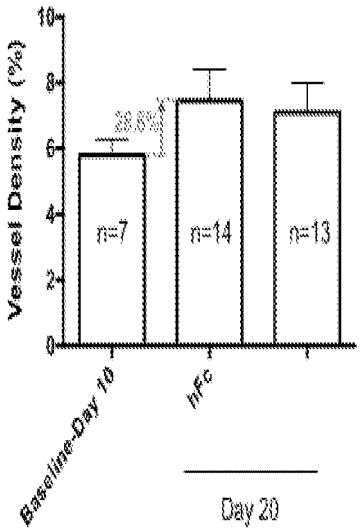


Figure 3 (Contd.)

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