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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/149,468	06/03/2014	8741963	PAT050279-US-CNT	1536

1095 7590 05/14/2014

NOVARTIS PHARMACEUTICAL CORPORATION
INTELLECTUAL PROPERTY DEPARTMENT
ONE HEALTH PLAZA 433/2
EAST HANOVER, NJ 07936-1080

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

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

The Patent Term Adjustment is 21 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

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

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

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

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

Peter C. Hiestand, Allschwil, SWITZERLAND;
Christian Schnell, Hesingue, FRANCE;

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	Spivack
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number / Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
/P.S./		US-2006/0046979	03-02-2006	Carolyn Ann Foster et al.	
		US-			
		US-			
		US-			
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		US-			
		US-			
		US-			
		US-			
		US-			

Change(s) applied to document, /A.E.M./ 3/27/2014

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document / Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
/P.S./		WO 2004/028521	04-08-~~2008~~	Novartis AG 2004		☐
/P.S./		WO 2003/099192	12-04-2003	Novartis AG		☐
/P.S./		WO 2003/097028	11-27-~~203~~ 2003	Novartis AG		☐
/P.S./		WO 2004/050073	06-17-2004	Doosan Corporation		☐
/P.S./		WO 2005/123104	12/2005			☐
						☐

Change(s) applied to document, /A.E.M./ 3/26/2014

Change(s) applied to document. /S.X.S./ 3/17/2014

Examiner Signature	/Phyllis Spivack/	Date Considered	03/24/2012

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1095 7590 01/21/2014
NOVARTIS PHARMACEUTICAL CORPORATION
INTELLECTUAL PROPERTY DEPARTMENT
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

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

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/149,468	05/31/2011	Peter C. Hiestand	PAT050279-US-CNT	1536

TITLE OF INVENTION: S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	04/21/2014

EXAMINER	ART UNIT	CLASS-SUBCLASS
WEDDINGTON, KEVIN E	1629	514-667000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

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

1 Andrew Holmes
2
3

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

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

(A) NAME OF ASSIGNEE: Novartis AG

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Basel, CH

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

4a. The following fee(s) are submitted:
- ☑ Issue Fee
- ☑ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 190134 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ☐ Applicant certifying micro entity status. See 37 CFR 1.29 — NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ☐ Applicant asserting small entity status. See 37 CFR 1.27 — NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ☐ Applicant changing to regular undiscounted fee status. — NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

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

Authorized Signature / Andrew Holmes / Date April 21, 2014

Typed or printed name Andrew Holmes Registration No. 51813

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Electronic Patent Application Fee Transmittal

Application Number:	13149468
Filing Date:	31-May-2011
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Filer:	Andrew K. Holmes/Angel Matos
Attorney Docket Number:	PAT050279-US-CNT

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl Issue Fee	1501	1	960	960
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**960**

Electronic Acknowledgement Receipt

EFS ID:	18814595
Application Number:	13149468
International Application Number:	
Confirmation Number:	1536
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Customer Number:	1095
Filer:	Andrew K. Holmes/Angel Matos
Filer Authorized By:	Andrew K. Holmes
Attorney Docket Number:	PAT050279-US-CNT
Receipt Date:	21-APR-2014
Filing Date:	31-MAY-2011
Time Stamp:	14:55:17
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$960
RAM confirmation Number	1125
Deposit Account	190134
Authorized User	

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	SIGNED_PAT050279-US-CNT-Issue-Fee-Trans.pdf	90796 458d49fcf74828ddbb254d364de5380e850bf772	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30328 bdb6837c1ca1bd1faab2924fbf12be490e3a9130	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	121124		

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

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

New International Application Filed with the USPTO as a Receiving Office
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Receipt date: 09/29/2011

13149468 - 1908 0829

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	Spivack
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
/P.S./		US-2006/0048979	03-02-2006	Carolyn Ann Foster et al.	
		US-			
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FOREIGN PATENT DOCUMENTS

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/P.S./		WO 2004/028521	04-08-2008	Novartis AG		☐
/P.S./		WO 2003/099192	12-04-2003	Novartis AG		☐
/P.S./		WO 2003/097028	11-27-203	Novartis AG		☐
/P.S./		WO 2004/050073	06-17-2004	Doosan Corporation		☐
/P.S./		WO 2005/123104				☐
						☐

Examiner Signature	/Phyllis Spivack/	Date Considered	03/24/2012

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Sheet 2 of 2

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Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	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[2]
		~~Mosshkowskij M.D. Pharmaceuticals, Moscow, Nowaja Wolna, 2001, in 2 volumes, vol. 1 p.11~~	☐
/P.S./		LaMontagne K „Antagonism of Sphingosine-1-Phosphate Receptors by FTY720 Inhibits Angiogenesis.." Cancer Research, Jan 2006, 66, 221-231.	☐
/P.S./		Hla. T. „Physiological and pathological actions of sphingosine 1-phosphate" Seminars in Cell & Developmental Biology, Oct 2004, 15(5), 513-520.	☐
/P.S./		Kappos L et al. „FTY720 in relapsing MS...." 23.06.2005 online (found 02.06.2011) URL:http://www.ms-in-europe.com/printversion/index.php?anr=105&cnr=4/>	☐
/P.S./		Ho J.W et al. „ Effects of a novel immunomodulating agent.." Molecular cancer theraputics, 2005 Set, 4(9), 1430-1438.	☐
/P.S./		Virely D.J. "Developing therapeutics for the treatment of multiple sclerosis." Journal of American Society for Experimental Neuro Therapeutics. Oct 2005, 2, 638-649. http://pubget.com/paper/16489371	☐
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/P.S./		WO 2005/123104				☐
						☐

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Art unit	
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	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[2]
		~~Mascbkowskij M.D. Pharmaceuticals, Moscow, Nowaja Wolna, 2001, in 2 volumes, vol.1 p.11~~	☐
/P.S./		LaMontagne K „Antagonism of Sphingosine-1-Phosphate Receptors by FTY720 Inhibits Angiogenesis.." Cancer Research, Jan 2006, 66, 221-231.	☐
/P.S./		Hla. T. „Physiological and pathological actions of sphingosine 1-phosphate" Seminars in Cell & Developmental Biology, Oct 2004, 15(5), 513-520.	☐
/P.S./		Kappos L et al. „FTY720 in relapsing MS...." 23.06.2005 online (found 02.06.2011) URL:http://www.ms-in-europe.com/printversion/index.php?anr=105&cnr=4/>	☐
/P.S./		Ho J.W et al. „ Effects of a novel immunomodulating agent.." Molecular cancer theraputics, 2005 Set, 4(9), 1430-1438.Found:	☐
/P.S./		Virely D.J. "Developing therapeutics for the treatment of multiple sclerosis." Journal of American Society for Experimental Neuro Therapeutics. Oct 2005, 2, 638-649. http://pubget.com/paper/16489371	☐
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Examiner Signature	/Phyllis Spivack/	Date Considered	03/24/2012

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[redacted]

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UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/149,468	05/31/2011	Peter C. Hiestand	PAT050279-US-CNT	1536

7590 03/24/2014

NOVARTIS PHARMACEUTICAL CORPORATION
INTELLECTUAL PROPERTY DEPARTMENT
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

EXAMINER	
WEDDINGTON, KEVIN E	
ART UNIT	PAPER NUMBER
1629	

DATE MAILED: 03/24/2014

PRIORITY ACKNOWLEDGMENT

☐ 1. Receipt is acknowledged of priority papers submitted under 35 U.S.C. 119. The papers have been placed of record in the file.

☑ 2. Applicant's claim for priority, based on papers filed in parent Application Number 12/303,765 submitted under 35 U.S.C. 119, is acknowledged.

☐ 3. The priority papers, submitted _____, after payment of the issue fee are

☐ acknowledged
While the priority claim or certified copy filed will be placed in the file record, neither will be reviewed and the patent when published will not include the priority claim.
See 37 CFR 1.55(a)(2).

☐ not acknowledged since the processing fee in 37 CFR 1.17(i) has not been received.

☐ 4. For utility and plant applications filed on or after November 29, 2000, the priority claim is not entered because the claim was not presented within the time limit required by 37 CFR 1.55(a)(1). A petition to accept a delayed claim for priority under 35 U.S.C. 119(a) - (d) or (f), or 365(a) may be filed. See 37 CFR 1.55(c) and MPEP 201.14(a).

for

571-272-4200 or 1-888-786-0101
Application Assistance Unit
Office of Data Management

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NOTICE OF ALLOWANCE AND FEE(S) DUE

1095 7590 01/21/2014

NOVARTIS PHARMACEUTICAL CORPORATION
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EXAMINER
WEDDINGTON, KEVIN E

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13/149,468	05/31/2011	Peter C. Hiestand	PAT050279-US-CNT	1536

TITLE OF INVENTION: S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	04/21/2014

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

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

HOW TO REPLY TO THIS NOTICE:

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

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

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

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

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

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

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

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

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

1095 7590 01/21/2014
NOVARTIS PHARMACEUTICAL CORPORATION
INTELLECTUAL PROPERTY DEPARTMENT
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

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

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

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/149,468	05/31/2011	Peter C. Hiestand	PAT050279-US-CNT	1536

TITLE OF INVENTION: S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	04/21/2014

EXAMINER	ART UNIT	CLASS-SUBCLASS
WEDDINGTON, KEVIN E	1629	514-667000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

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

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

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

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

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

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29
  NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27
  NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status.
  NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

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

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
WEDDINGTON, KEVIN E	

ART UNIT	PAPER NUMBER
1629	

DATE MAILED: 01/21/2014

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)

(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 78 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 78 day(s).

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

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

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

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

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

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

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

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

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

Notice of Allowability	Application No. 13/149,468	Applicant(s) HIESTAND ET AL.	
	Examiner KEVIN E. WEDDINGTON	Art Unit 1629	AIA (First Inventor to File) Status No

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

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

1. ☒ This communication is responsive to *December 30, 2013*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *12-14, 16, 17, 21-23 and 25; renumbered respectively 1, 7, 2, 3, 9, 8 and 4-6*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

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

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

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☒ Interview Summary (PTO-413), Paper No./Mail Date *12/30/2013* .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/KEVIN E WEDDINGTON/
Primary Examiner, Art Unit 1629

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

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Mr. Andrew Holmes on December 30, 2013.

The application has been amended as follows:

In the Claims:

Cancel claim 24.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to KEVIN E. WEDDINGTON whose telephone number is (571)272-0587. The examiner can normally be reached on 12:30 pm -9:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffery S. Lundgren can be reached on (571)272-5541. 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.

KEVIN E WEDDINGTON
Primary Examiner
Art Unit 1629

/KEVIN E WEDDINGTON/
Primary Examiner, Art Unit 1629

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	13/149,468	HIESTAND ET AL.	
	Examiner	Art Unit	
	KEVIN E. WEDDINGTON	1629	

All participants (applicant, applicant's representative, PTO personnel):

(1) *KEVIN E. WEDDINGTON*. (3)_____.

(2) *Andrew Holmes*. (4)_____.

Date of Interview: *30 December 2013*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *Claim 24*.

Identification of prior art discussed: *NONE*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

The attorney of record, Mr. Holmes, authorized the cancellation of claim 24.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/KEVIN E WEDDINGTON/ Primary Examiner, Art Unit 1629	

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13149468	HIESTAND ET AL.
	Examiner	Art Unit
	KEVIN E WEDDINGTON	1629

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

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

CLAIM		DATE								
Final	Original	09/24/2013	12/30/2013							
	1	-	-							
	2	-	-							
	3	-	-							
	4	-	-							
	5	-	-							
	6	-	-							
	7	-	-							
	8	-	-							
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	18	-	-							
	19	-	-							
	20	-	-							
8	21	=	=							
4	22	=	=							
5	23	=	=							
	24	✓	-							
6	25	=	=							

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13149468	HIESTAND ET AL.
	Examiner	Art Unit
	KEVIN E WEDDINGTON	1629

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
514	667	9/24/2013	KEW
514	903	9/24/2013	KEW

SEARCH NOTES		
Search Notes	Date	Examiner
CAS-ONLINE search with REGISTRY and USPATALL	9/24/2013	KEW
EAST and PALM for Inventors' Names for Related Applications for ODP	9/24/2013	KEW
Updated CAS-ONLINE search with REGISTRY and USPATALL	12/30/2013	KEW
Updated EAST and PALM for Inventors' Names for Related Applications for ODP	12/30/2013	KEW

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
514	667	12/30/2013	KEW
514	903	12/30/2013	KEW

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BIB DATA SHEET

CONFIRMATION NO. 1536

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
13/149,468	05/31/2011	514	1629	PAT050279-US-CNT

APPLICANTS

INVENTORS

Peter C. Hiestand, Allschwil, SWITZERLAND;
Christian Schnell, Hesingue, FRANCE;

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

This application is a CON of 12/303,765 12/08/2008 ABN
which is a 371 of PCT/EP2007/005597 06/25/2007

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

UNITED KINGDOM 0612721.1 06/27/2006

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

06/15/2011

Foreign Priority claimed	☑ Yes ☐ No	☐ Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	☑ Yes ☐ No		SWITZERLAND	0	10	3
Verified and Acknowledged	/KEVIN E WEDDINGTON/ Examiner's Signature	Initials				

ADDRESS

NOVARTIS PHARMACEUTICAL CORPORATION
INTELLECTUAL PROPERTY DEPARTMENT
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080
UNITED STATES

TITLE

S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS

FILING FEE RECEIVED		
1480	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

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13149468	HIESTAND ET AL.
	Examiner	**Art Unit**
	KEVIN E WEDDINGTON	1629

CPC		
Symbol	**Type**	**Version**

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

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	9	
/KEVIN E WEDDINGTON/ Primary Examiner.Art Unit 1629	12/30/2013	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13149468	HIESTAND ET AL.
	Examiner	**Art Unit**
	KEVIN E WEDDINGTON	1629

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION										
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED					
514	667	A	6	1	K	31 / 13 (2006.01.01)						

CROSS REFERENCE(S)					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				
514	903				

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	9	
/KEVIN E WEDDINGTON/ Primary Examiner.Art Unit 1629	12/30/2013	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13149468	HIESTAND ET AL.
	Examiner	**Art Unit**
	KEVIN E WEDDINGTON	1629

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

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
	1	9	17												
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	11														
1	12														
7	13														
2	14														
	15														
3	16														

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	9	
/KEVIN E WEDDINGTON/ Primary Examiner.Art Unit 1629	12/30/2013	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/149,468	05/31/2011	Peter C. Hiestand	PAT050279-US-CNT	1536

1095 7590 09/30/2013

NOVARTIS PHARMACEUTICAL CORPORATION
INTELLECTUAL PROPERTY DEPARTMENT
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

EXAMINER	
WEDDINGTON, KEVIN E	

ART UNIT	PAPER NUMBER
1629	

NOTIFICATION DATE	DELIVERY MODE
09/30/2013	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):

php.patents@novartis.com

Office Action Summary	Application No. 13/149,468	Applicant(s) HIESTAND ET AL.	
	Examiner KEVIN E. WEDDINGTON	Art Unit 1629	AIA (First Inventor to File) Status No

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

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, 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 *February 18, 2013*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

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

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *12-14,16,17 and 21-25* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☒ Claim(s) *12-14,16,17,21-23 and 25* is/are allowed.

7)☒ Claim(s) *24* 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 _____ 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/08) Paper No(s)/Mail Date *4/18/13*.

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

4) ☐ Other: _____.

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

Claims 12-14, 16, 17 and 21-25 are presented for examination.

Applicants' request for continued examination, amendment and response filed February 18, 2013; and the information disclosure statement filed April 18, 2013 have been received and entered.

Accordingly, the rejection made under 35 USC 103(a) as being unpatentable over Virley, D.D., Journal of the American Society for Experimental NeuroTherapeutics, in view of LaMontagne et al., Cancer Research, and further in view of Kovarik et al., WO 06/058316 as set forth in the previous Office action dated November 16, 2012 at pages 2-3 as applied to claims 12-21 is hereby withdrawn because the applicants amendment the independent claims and the prior art does not read on applicants' newly limitation.

Allowable Subject Matter

Claims 12-14, 16, 17, 21-23 and 25 are allowable.

Claim Rejections - 35 USC § 112

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.

Claim 24 is 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.

Claim 24 depends on a cancelled claim 15.

Claim 24 is not allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to KEVIN E. WEDDINGTON whose telephone number is (571)272-0587. The examiner can normally be reached on 12:30 pm -9:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffery S. Lundgren can be reached on (571)272-5541. 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.

KEVIN E WEDDINGTON
Primary Examiner
Art Unit 1629

/KEVIN E WEDDINGTON/
Primary Examiner, Art Unit 1629

PTO/SB/08a (07-09)
PTO/SB/08b (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	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[2]
/K.W./		K. Rammohan et al, Poster on 'Long-Term Safety of Fingolimod in Patients with Relapsing-Remitting Multiple Sclerosis: Results from Phase 3 FREEDOMS II Extension Study' March 16-23 2013, San Diego, US, 65th American Academy of Neurology Annual Meeting.	☐
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Examiner Signature	/Kevin Weddington/	**Date Considered**	09/24/2013

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

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13149468	HIESTAND ET AL.
	Examiner	Art Unit
	KEVIN E WEDDINGTON	1629

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

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

CLAIM		DATE								
Final	Original	09/24/2013								
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	23	=								
	24	✓								
	25	=								

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                  Now Includes 2013 MeSH Vocabulary and New Fields
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 NEWS  9  JAN 31  INPAFAMDB Enhanced with Patent Family Counts
 NEWS 10  FEB  6  Enhancements to COMPENDEX
 NEWS 11  FEB 22  2013 MARPAT Backfile Expansion Update
 NEWS 12  MAR 06  Derwent World Patents Index (DWPI) New Coverage - Indonesia
 NEWS 13  MAR 11  JAPIO Will No Longer Be Updated from March 2013 Onwards
 NEWS 14  MAR 22  Cooperative Patent Classification (CPC) Added to USPATOLD
                  on STN
 NEWS 15  MAR 25  SciSearch on STN Now Includes New Fields
                  Find Grant Information More Easily
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                  and Optimized for Use as a Companion Database for Embase
 NEWS 17  APR 30  Derwent WPI: The New Cooperative Patent Classification Is
                  Now Available
 NEWS 18  MAY 21  STN Updated to Reflect Streamlining of CAS Roles
 NEWS 19  MAY 24  CABA Has Been Reloaded on May 24, 2013
 NEWS 20  MAY 28  STN Adds Indian Patent Full Text File - INFULL
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DICTIONARY FILE UPDATES:  23 SEP 2013  HIGHEST RN 1453165-12-2

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=> e fty720/cn
E1            1     FTY 72O/CN
E2            1     FTY-P/CN
E3            0 --> FTY720/CN
E4            1     FTYR/CN
E5            1     FTZ-F1 (ACANTHOPAGRUS SCHLEGELI GENE FF1A N-TERMINAL FRAGMEN
                    T)/CN
E6            1     FTZ-F1 (ACANTHOPAGRUS SCHLEGELI GENE FF1B N-TERMINAL FRAGMEN
                    T)/CN
E7            1     FTZ-F1 (RANA RUGOSA ERYTHROCYTE GENE FTZ-F1)/CN
E8            1     FTZ-F1 (SEBASTES SCHLEGELII GENE FTZ-F1)/CN
E9            1     FTZ-F1 .BETA. (RANA RUGOSA TESTIS GENE FTZ-F1 ISOFORM .BETA.
                    )/CN
E10           1     FTZ-F1 HOMOLOG (ORANGE-SPOTTED GROUPER PITUITARY)/CN
E11           1     FTZ-F1-LIKE PROTEIN (RANA RUGOSA GENE FTZ-F1 ISOFORM .ALPHA.
                    )/CN
E12           1     FTZ-F1-LIKE PROTEIN (RANA RUGOSA GENE FTZ-F1 ISOFORM .BETA.)
                    /CN

=> s e1
L1            1 "FTY 72O"/CN

=> d

L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2013 ACS on STN
RN   162359-56-0  REGISTRY
ED   Entered STN:  21 Apr 1995
CN   1,3-Propanediol, 2-amino-2-[2-(4-octylphenyl)ethyl]-, hydrochloride (1:1)
     (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   1,3-Propanediol, 2-amino-2-[2-(4-octylphenyl)ethyl]-, hydrochloride (9CI)
OTHER NAMES:
CN   2-Amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride
CN   Fingolimod hydrochloride
CN   FTY 720
CN   FTY 72O
CN   Gilenya
CN   Imusera
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MF   C19 H33 N O2 . Cl H
CI   COM
SR   CA
LC   STN Files:   ADISINSIGHT, ADISNEWS, ANABSTR, BIOSIS, BIOTECHNO, CA,
       CAPLUS, CASREACT, CBNB, CHEMCATS, EMBASE, IMSPATENTS, IMSRESEARCH, IPA,
       PATDPASPC, RTECS*, TOXCENTER, USAN, USPAT2, USPATFULL
         (*File contains numerically searchable property data)
CRN  (162359-55-9)
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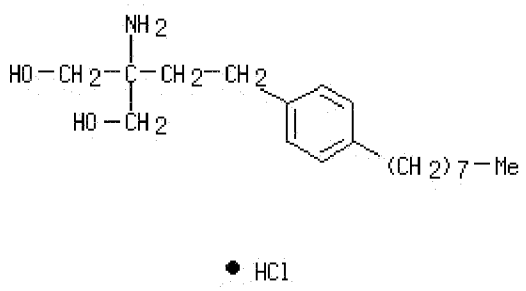


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**PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT**

             970 REFERENCES IN FILE CA (1907 TO DATE)
              16 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
             980 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> file uspatall
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       9.89      10.13

FILE 'USPATFULL' ENTERED AT 20:25:37 ON 24 SEP 2013
CA INDEXING COPYRIGHT (C) 2013 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'USPATOLD' ENTERED AT 20:25:37 ON 24 SEP 2013
CA INDEXING COPYRIGHT (C) 2013 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'USPAT2' ENTERED AT 20:25:37 ON 24 SEP 2013
CA INDEXING COPYRIGHT (C) 2013 AMERICAN CHEMICAL SOCIETY (ACS)

=> d his

     (FILE 'HOME' ENTERED AT 20:23:31 ON 24 SEP 2013)

     FILE 'REGISTRY' ENTERED AT 20:23:36 ON 24 SEP 2013
                E FTY720/CN
L1            1 S E1

     FILE 'USPATFULL, USPATOLD, USPAT2' ENTERED AT 20:25:37 ON 24 SEP 2013

=> s l1
L2          256 L1

=> d 200-256

L2   ANSWER 200 OF 256  USPATFULL on STN
Full Text
AN     1998:17339  USPATFULL
TI     2-amino-1,3-propanediol compound and immunosuppressant
IN     Fujita, Tetsuro, Kyoto, Japan
       Sasaki, Shigeo, Hyogo, Japan
       Yoneta, Masahiko, Hyogo, Japan
       Mishina, Tadashi, Saitama, Japan
       Adachi, Kunitomo, Saitama, Japan
       Chiba, Kenji, Saitama, Japan
PA     Yoshitomi Pharmaceutical Industries, Ltd., Osaka, Japan (non-U.S.
```

corporation)
Taito Co., Ltd., Tokyo, Japan (non-U.S. corporation)
PI US 5719176 19980217
AI US 1996-725890 19961002 (8)
RLI Division of Ser. No. US 1994-244942, filed on 17 Jun 1994, now patented, Pat. No. US 5604229
PRAI JP 1992-283281 19921021
JP 1993-179427 19930720
DT Utility
FS Granted
LN.CNT 10472
INCL INCLM: 514/440.000
INCLS: 514/459.000; 514/471.000; 514/561.000; 514/564.000; 564/123.000; 564/336.000; 564/340.000; 564/342.000; 564/346.000; 564/374.000; 564/383.000; 564/459.000
NCL NCLM: 514/440.000
NCLS: 514/459.000; 514/471.000; 514/561.000; 514/564.000; 564/123.000; 564/336.000; 564/340.000; 564/342.000; 564/346.000; 564/374.000; 564/383.000; 564/459.000
CPC CPCI C07D0207-325 [I]; C07C0215-10 [I]; C07C0215-18 [I]; C07C0215-20 [I]; C07C0215-24 [I]; C07C0215-26 [I]; C07C0215-28 [I]; C07C0215-34 [I]; C07C0215-42 [I]; C07C0215-54 [I]; C07C0215-56 [I]; C07C0215-68 [I]; C07C0217-28 [I]; C07C0217-30 [I]; C07C0217-34 [I]; C07C0217-48 [I]; C07C0217-52 [I]; C07C0217-64 [I]; C07C0217-72 [I]; C07C0217-76 [I]; C07C0219-06 [I]; C07C0219-24 [I]; C07C0219-30 [I]; C07C0225-04 [I]; C07C0225-06 [I]; C07C0225-10 [I]; C07C0225-14 [I]; C07C0225-16 [I]; C07C0229-22 [I]; C07C0229-38 [I]; C07C0229-46 [I]; C07C0233-18 [I]; C07C0233-31 [I]; C07C0233-35 [I]; C07C0233-36 [I]; C07C0233-41 [I]; C07C0233-43 [I]; C07C0233-47 [I]; C07C0237-06 [I]; C07C0237-24 [I]; C07C0237-30 [I]; C07C0251-38 [I]; C07C0271-20 [I]; C07C0271-24 [I]; C07C0271-28 [I]; C07C0323-25 [I]; C07C0323-30 [I]; C07C0323-32 [I]; C07C0323-34 [I]; C07C0323-41 [I]; C07C2101-08; C07C2101-14; C07D0207-06 [I]; C07D0207-09 [I]; C07D0207-335 [I]; C07D0211-14 [I]; C07D0211-26 [I]; C07D0213-38 [I]; C07D0213-40 [I]; C07D0231-12 [I]; C07D0233-56 [I]; C07D0233-64 [I]; C07D0237-08 [I]; C07D0239-26 [I]; C07D0241-12 [I]; C07D0249-08 [I]; C07D0261-08 [I]; C07D0263-32 [I]; C07D0265-30 [I]; C07D0275-02 [I]; C07D0277-28 [I]; C07D0279-12 [I]; C07D0295-13 [I]; C07D0295-135 [I]; C07D0307-52 [I]; C07D0309-32 [I]; C07D0317-28 [I]; C07D0333-20 [I]
IPC [6]
IPCI C07C0711-00 [ICM,6]; A01N0043-40 [ICS,6]; A01N0033-02 [ICS,6]
IPCR A01N0033-02 [I]; A01N0043-40 [I]; A01N0043-60 [I]; A01N0057-00 [I]; C07C0215-10 [I]; C07C0215-18 [I]; C07C0215-20 [I]; C07C0215-24 [I]; C07C0215-26 [I]; C07C0215-28 [I]; C07C0215-34 [I]; C07C0215-42 [I]; C07C0215-54 [I]; C07C0215-56 [I]; C07C0215-68 [I]; C07C0217-28 [I]; C07C0217-30 [I]; C07C0217-34 [I]; C07C0217-48 [I]; C07C0217-52 [I]; C07C0217-64 [I]; C07C0217-72 [I]; C07C0217-76 [I]; C07C0219-06 [I]; C07C0219-24 [I]; C07C0219-30 [I]; C07C0225-04 [I]; C07C0225-06 [I]; C07C0225-10 [I]; C07C0225-14 [I]; C07C0225-16 [I]; C07C0229-22 [I]; C07C0229-38 [I]; C07C0229-46 [I]; C07C0233-18 [I]; C07C0233-31 [I]; C07C0233-35 [I]; C07C0233-36 [I]; C07C0233-41 [I]; C07C0233-43 [I]; C07C0233-47 [I]; C07C0237-06 [I]; C07C0237-24 [I]; C07C0237-30 [I]; C07C0251-38 [I]; C07C0271-20 [I]; C07C0271-24 [I]; C07C0271-28 [I]; C07C0323-25 [I]; C07C0323-30 [I]; C07C0323-32 [I]; C07C0323-34 [I]; C07C0323-41 [I]; C07D0207-06 [I]; C07D0207-09 [I]; C07D0207-32 [N]; C07D0207-325 [I]; C07D0207-335 [I]; C07D0211-14 [I]; C07D0211-26 [I]; C07D0213-38 [I]; C07D0213-40 [I]; C07D0233-54 [I]; C07D0237-08 [I]; C07D0239-26 [I]; C07D0241-12 [I]; C07D0261-08 [I]; C07D0263-32 [I]; C07D0265-30 [I]; C07D0275-02 [I]; C07D0277-28 [I]; C07D0279-12 [I]; C07D0295-13 [I]; C07D0295-135 [I]; C07D0307-52 [I]; C07D0309-32 [I]; C07D0317-28 [I]; C07D0333-20 [I]; C07D0521-00 [I]
EXF 514/440; 514/459; 514/471; 514/561; 514/564; 564/336; 564/340; 564/342; 564/346; 564/374; 564/383; 564/459; 564/123
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 201 OF 256 USPATFULL on STN

Full Text
AN 97:14705 USPATFULL
TI 2-amino-1,3-propanediol compound and immunosuppressant
IN Fujita, Tetsuro, Muko, Japan
Sasaki, Shigeo, Kobe, Japan
Yoneta, Masahiko, Kobe, Japan
Mishina, Tadashi, Iruma, Japan
Adachi, Kunitomo, Iruma, Japan
Chiba, Kenji, Iruma, Japan
PA Yoshitomi Pharmaceutical Industries, Ltd., Osaka, Japan (non-U.S. corporation)
Taito Co., Ltd., Tokyo, Japan (non-U.S. corporation)
PI US 5604229 19970218
WO 9408943 19940428
AI US 1994-244942 19940617 (8)
WO 1993-JP1515 19931018
19940617 PCT 371 date
19940617 PCT 102(e) date
PRAI JP 1992-283281 19921021
JP 1993-179427 19930720
DT Utility
FS Granted
LN.CNT 10702
INCL INCLM: 514/255.000
INCLS: 514/357.000; 514/372.000; 514/403.000; 514/427.000; 514/438.000; 514/459.000; 514/471.000; 544/401.000; 546/246.000; 546/247.000; 546/334.000; 548/214.000; 548/373.100; 548/516.000; 549/075.000; 549/426.000; 549/495.000; 549/472.000; 564/336.000; 564/340.000; 564/342.000; 564/346.000; 564/374.000; 564/383.000; 564/454.000; 564/123.000
NCL NCLM: 514/252.100
NCLS: 514/357.000; 514/372.000; 514/403.000; 514/427.000; 514/438.000; 514/459.000; 514/471.000; 544/401.000; 546/246.000; 546/247.000; 546/334.000; 548/214.000; 548/373.100; 548/516.000; 549/075.000; 549/426.000; 549/472.000; 549/495.000; 564/123.000; 564/336.000; 564/340.000; 564/342.000; 564/346.000; 564/374.000; 564/383.000; 564/454.000
CPC CPCI C07D0207-325 [I]; C07C0215-10 [I]; C07C0215-18 [I]; C07C0215-20 [I]; C07C0215-24 [I]; C07C0215-26 [I]; C07C0215-28 [I]; C07C0215-34 [I]; C07C0215-42 [I]; C07C0215-54 [I]; C07C0215-56 [I]; C07C0215-68 [I]; C07C0217-28 [I]; C07C0217-30 [I]; C07C0217-34 [I]; C07C0217-48 [I]; C07C0217-52 [I]; C07C0217-64 [I]; C07C0217-72 [I]; C07C0217-76 [I]; C07C0219-06 [I]; C07C0219-24 [I]; C07C0219-30 [I]; C07C0225-04 [I]; C07C0225-06 [I]; C07C0225-10 [I]; C07C0225-14 [I]; C07C0225-16 [I]; C07C0229-22 [I]; C07C0229-38 [I]; C07C0229-46 [I]; C07C0233-18 [I]; C07C0233-31 [I]; C07C0233-35 [I]; C07C0233-36 [I]; C07C0233-41 [I]; C07C0233-43 [I]; C07C0233-47 [I]; C07C0237-06 [I]; C07C0237-24 [I]; C07C0237-30 [I]; C07C0251-38 [I]; C07C0271-20 [I]; C07C0271-24 [I]; C07C0271-28 [I]; C07C0323-25 [I]; C07C0323-30 [I]; C07C0323-32 [I]; C07C0323-34 [I]; C07C0323-41 [I]; C07C2101-08; C07C2101-14; C07D0207-06 [I]; C07D0207-09 [I]; C07D0207-335 [I]; C07D0211-14 [I]; C07D0211-26 [I]; C07D0213-38 [I]; C07D0213-40 [I]; C07D0231-12 [I]; C07D0233-56 [I]; C07D0233-64 [I]; C07D0237-08 [I]; C07D0239-26 [I]; C07D0241-12 [I]; C07D0249-08 [I]; C07D0261-08 [I]; C07D0263-32 [I]; C07D0265-30 [I]; C07D0275-02 [I]; C07D0277-28 [I]; C07D0279-12 [I]; C07D0295-13 [I]; C07D0295-135 [I]; C07D0307-52 [I]; C07D0309-32 [I]; C07D0317-28 [I]; C07D0333-20 [I]
IPC [6]
IPCI C07C0211-00 [ICM,6]; C07D0333-20 [ICS,6]; A01N0043-40 [ICS,6]; A01N0033-02 [ICS,6]
IPCR A01N0033-02 [I]; A01N0043-40 [I]; A01N0043-60 [I]; A01N0057-00 [I]; C07C0215-10 [I]; C07C0215-18 [I]; C07C0215-20 [I]; C07C0215-24 [I]; C07C0215-26 [I]; C07C0215-28 [I]; C07C0215-34 [I]; C07C0215-42 [I]; C07C0215-54 [I]; C07C0215-56 [I]; C07C0215-68 [I]; C07C0217-28 [I]; C07C0217-30 [I]; C07C0217-34 [I]; C07C0217-48 [I]; C07C0217-52 [I]; C07C0217-64 [I]; C07C0217-72 [I]; C07C0217-76 [I]; C07C0219-06 [I]; C07C0219-24 [I]; C07C0219-30 [I]; C07C0225-04 [I]; C07C0225-06 [I]; C07C0225-10 [I]; C07C0225-14 [I]; C07C0225-16 [I]; C07C0229-22

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EXF 546/210; 546/246; 546/247; 546/334; 560/172; 562/11; 558/169; 514/114; 514/119; 514/357; 514/255; 514/372; 514/403; 514/427; 514/438; 514/459; 514/471; 564/336; 564/340; 564/342; 564/346; 564/374; 564/383; 564/454; 564/123; 549/75; 549/426; 549/495; 548/214; 548/373.1; 548/516; 544/401

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 202 OF 256 USPAT2 on STN

Full Text

AN 2012:324839 USPAT2

TI Compounds that modulate intracellular calcium

IN Velicelebi, Gonul, San Diego, CA, UNITED STATES
Stauderman, Kenneth A., San Diego, CA, UNITED STATES
Pleynet, David P. M., Auburndale, MA, UNITED STATES
Cheng, Soan, San Diego, CA, UNITED STATES
Whitten, Jeffrey P., Santee, CA, UNITED STATES

PA Calcimedica, Inc., La Jolla, CA, UNITED STATES (U.S. corporation)

PI US 8524765 B2 20130903

AI US 2012-553726 20120719 (13)

RLI Division of Ser. No. US 2008-192812, filed on 15 Aug 2008, Pat. No. US 8263641

PRAI US 2007-971161P 20070910 (60)

DT Utility

FS GRANTED

LN.CNT 6498

INCL INCLM: 514/447.000
INCLS: 549/069.000

NCL NCLM: 514/443.000
NCLS: 514/447.000

CPC CPCI C07D0333-68 [I]; A61K0031-00 [I]; A61K0031-405 [I]
CPCI-2 C07D0333-68 [I]; A61K0031-00 [I]; A61K0031-405 [I]

IPC IPCI A61K0031-381 [I]; A61P0013-12 [I]; A61P0027-02 [I]; A61P0001-16 [I]; A61P0011-00 [I]; A61P0019-02 [I]; A61P0017-06 [I]; A61P0001-00 [I]; A61P0009-00 [I]; A61P0017-00 [I]; A61P0021-00 [I]; A61P0011-02 [I]; A61P0015-02 [I]; A61P0019-10 [I]; A61P0037-06 [I]; A61P0003-10 [I]; A61P0037-00 [I]; A61P0021-04 [I]; A61P0007-06 [I]; A61P0011-06 [I]; A61P0025-00 [I]; A61P0029-00 [I]
IPCI-2 A61K0031-381 [I]; C07D0333-40 [I]
IPCR A61K0031-381 [I]; A61P0001-00 [I]; A61P0001-16 [I]; A61P0003-10 [I]; A61P0007-06 [I]; A61P0009-00 [I]; A61P0011-00 [I]; A61P0011-02 [I]; A61P0011-06 [I]; A61P0013-12 [I]; A61P0015-02 [I]; A61P0017-00 [I]; A61P0017-06 [I]; A61P0019-02 [I]; A61P0019-10 [I]; A61P0021-00 [I]; A61P0021-04 [I]; A61P0025-00 [I]; A61P0027-02 [I]; A61P0029-00 [I]; A61P0037-00 [I]; A61P0037-06 [I]

EXF 514/447; 549/69

L2 ANSWER 203 OF 256 USPAT2 on STN

Full Text

AN 2012:295859 USPAT2

TI Methods for modulating inflammatory and/or immune responses

IN Marshall, Vivienne S., San Antonio, TX, UNITED STATES
Banas, Richard A., Turtle Creek, PA, UNITED STATES
Trumpower, Catherine J., Pittsburgh, PA, UNITED STATES

PA Stemnion, Inc., Pittsburgh, PA, UNITED STATES (U.S. corporation)

PI US 8506949 B2 20130813

AI US 2012-483369 20120530 (13)

RLI Division of Ser. No. US 2009-448903, filed on 13 Jul 2009, Pat. No. US

8221741
PRAI US 2007-880745P 20070117 (60)
US 2007-902440P 20070221 (60)
US 2007-997604P 20071004 (60)
DT Utility
FS GRANTED
LN.CNT 1982
INCL INCLM: 424/093.210
INCLS: 424/093.700; 424/184.100; 424/141.100; 424/173.100
NCL NCLM: 424/093.210; 424/093.700
NCLS: 424/093.700; 424/141.100; 424/173.100; 424/184.100; 424/572.000; 435/002.000
CPC CPCI A61K0039-001 [I]; A61K0035-50 [I]; A61K2039-5158; C12N0005-0634 [I]; C12N2502-025
CPCI-2 A61K0039-001 [I]; A61K0035-50 [I]; A61K2039-5158; C12N0005-0634 [I]; C12N2502-025
IPC IPCI A61K0035-12 [I]; A61P0029-00 [I]; C12N0005-078 [I]
IPCI-2 A61K0039-00 [I]; A61K0035-12 [I]; A61K0038-21 [I]
IPCR A61K0035-12 [I]; A61P0029-00 [I]; C12N0005-078 [I]

L2 ANSWER 204 OF 256 USPAT2 on STN
Full Text
AN 2012:220564 USPAT2
TI Treatment for multiple sclerosis
IN Lukashev, Matvey E., Tewksbury, MA, UNITED STATES
O'Neill, Gilmore, Medford, MA, UNITED STATES
PA Biogen Idec MA Inc., Cambridge, MA, UNITED STATES (U.S. corporation)
PI US 8399514 B2 20130319
AI US 2012-372426 20120213 (13)
RLI Continuation of Ser. No. US 1900-526296, ABANDONED A 371 of International Ser. No. WO 2008-US1602, filed on 7 Feb 2008
PRAI US 2007-888921P 20070208 (60)
DT Utility
FS GRANTED
LN.CNT 2560
INCL INCLM: 514/549.000
NCL NCLM: 514/549.000; 514/547.000
CPC CPCI G01N0033-502 [I]; G01N2333-90209; G01N2800-285
CPCI-2 G01N0033-502 [I]; G01N2333-90209; G01N2800-285
IPC IPCI A61K0031-225 [I]; A61P0025-00 [I]
IPCI-2 A61K0031-22 [I]
IPCR A61K0031-22 [I]

L2 ANSWER 205 OF 256 USPAT2 on STN
Full Text
AN 2012:152949 USPAT2
TI FTY720-derived anticancer agents
IN Chen, Ching-Shih, Upper Arlington, OH, UNITED STATES
Kulp, Samuel K., Hillard, OH, UNITED STATES
Wang, Dasheng, Dublin, OH, UNITED STATES
Byrd, John C., Columbus, OH, UNITED STATES
Muthusamy, Natarajan, Galloway, OH, UNITED STATES
PA The Ohio State University Research Foundation, Columbus, OH, UNITED STATES (U.S. corporation)
PI US 8309768 B2 20121113
AI US 2011-305927 20111129 (13)
PRAI US 2010-417566P 20101129 (61)
DT Utility
FS GRANTED
LN.CNT 2578
INCL INCLM: 564/355.000
INCLS: 564/336.000; 564/347.000
NCL NCLM: 564/355.000; 514/653.000
NCLS: 564/336.000; 564/347.000; 564/374.000
CPC CPCI C07C0217-74 [I]; C07C0217-64 [I]; C07C2101-02
CPCI-2 C07C0217-74 [I]; C07C0217-64 [I]; C07C2101-02
IPC IPCI A61K0031-137 [I]; A61P0035-00 [I]; C07C0215-28 [I]
IPCI-2 C07C0215-00 [I]; C07C0211-00 [I]; C07C0213-00 [I]
IPCR C07C0215-00 [I]; C07C0211-00 [I]; C07C0213-00 [I]
EXF 564/336; 564/347; 564/355

L2 ANSWER 206 OF 256 USPAT2 on STN

Full Text
AN 2011:348766 USPAT2
TI Tricyclic compounds
IN Wishart, Neil, Jefferson, MA, UNITED STATES
Frank, Kristine E., Worcester, MA, UNITED STATES
Friedman, Michael, Brookline, MA, UNITED STATES
George, Dawn M., Charlton, MA, UNITED STATES
Stewart, Kent D., Gurnee, IL, UNITED STATES
Wallace, Grier A., Sterling, MA, UNITED STATES
PA Abbott Laboratories, Abbott Park, IL, UNITED STATES (U.S. corporation)
PI US 8426411 B2 20130423
AI US 2010-958115 20101201 (12)
PRAI US 2009-265563P 20091201 (61)
US 2010-364116P 20100714 (61)
DT Utility
FS GRANTED
LN.CNT 22219
INCL INCLM: 514/250.000
NCL NCLM: 514/250.000; 424/085.200
NCLS: 424/085.100; 424/085.400; 424/085.500; 424/085.600; 424/085.700; 424/142.100; 424/158.100; 514/008.200; 514/009.100; 514/171.000; 514/210.210; 514/293.000; 544/346.000; 546/082.000
CPC CPCI C07D0487-14 [I]; A61K0031-437 [I]; A61K0031-4985 [I]; A61K0045-06 [I]; C07D0471-14 [I]; C07D0498-14 [I]; C07D0513-14 [I]
CPCI-2 C07D0487-14 [I]; A61K0031-437 [I]; A61K0031-4985 [I]; A61K0045-06 [I]; C07D0471-14 [I]; C07D0498-14 [I]; C07D0513-14 [I]
IPC IPCI A61K0031-4985 [I]; C07D0471-14 [I]; A61K0031-437 [I]; A61K0039-395 [I]; A61P0025-00 [I]; A61K0038-21 [I]; A61K0038-18 [I]; A61K0031-573 [I]; A61P0035-00 [I]; A61P0037-06 [I]; C07D0487-14 [I]; A61K0038-20 [I]
IPCI-2 A61K0031-50 [I]
IPCR A61K0031-4985 [I]; A61K0031-437 [I]; A61K0031-573 [I]; A61K0038-18 [I]; A61K0038-20 [I]; A61K0038-21 [I]; A61K0039-395 [I]; A61P0025-00 [I]; A61P0035-00 [I]; A61P0037-06 [I]; C07D0471-14 [I]; C07D0487-14 [I]
EXF 514/250

L2 ANSWER 207 OF 256 USPAT2 on STN
Full Text
AN 2011:328619 USPAT2
TI COMPOSITION AND METHOD FOR TREATMENT OF REPERFUSION INJURY AND TISSUE DAMAGE
IN Heffernan, Mark, Dublin 8, IRELAND
O'Neill, Luke, Dublin, IRELAND
McGuirk, Peter, Dublin, IRELAND
Keogh, Brian, Dublin, IRELAND
Locher, Christopher, Lexington, MA, UNITED STATES
De Kleijn, Dominique, Utrecht, NETHERLANDS
Arslan, Fatih, Utrecht, NETHERLANDS
Pasterkamp, Gerard, Utrecht, NETHERLANDS
PA Opsona Therapeutics Ltd, Dublin 8, IRELAND (non-U.S. corporation)
PI US 20120141466 A9 20120607
AI US 2011-671810 A1 20080804 (12)
WO 2008-EP60249 20080804
20110816 PCT 371 date
PRAI IE 2007-558 20070803
US 2008-38555P 20080321 (61)
DT Utility
FS APPLICATION
LN.CNT 3095
INCL INCLM: 424/133.100
INCLS: 435/375.000; 424/172.100; 424/143.100; 424/139.100; 424/158.100
NCL NCLM: 424/133.100
NCLS: 424/139.100; 424/143.100; 424/158.100; 424/172.100; 435/375.000
CPC CPCI A61K0045-06 [I]; A61K0031-00 [I]; A61K0031-7088 [I]; A61K0038-177 [I]; C07K0016-2896 [I]; C12N0015-1138 [I]; G01N0033-567 [I]; G01N2500-00
CPCI-2 A61K0045-06 [I]; A61K0031-00 [I]; A61K0031-7088 [I]; A61K0038-177 [I]; C07K0016-2896 [I]; C12N0015-1138 [I]; G01N0033-567 [I]; G01N2500-00
IPC IPCI A61K0039-395 [I]; A61P0039-06 [I]; A61P0007-02 [I]; A61P0009-00 [I]; A61P0009-10 [I]; A61P0009-06 [I]; A61P0009-04 [I];

A61P0009-12 [I]; C12N0005-071 [I]; A61P0007-10 [I]
IPCI-2 A61K0039-395 [I]; A61P0039-06 [I]; A61P0007-02 [I]; A61P0009-00 [I]; A61P0009-10 [I]; A61P0009-06 [I]; A61P0009-04 [I]; A61P0009-12 [I]; C12N0005-071 [I]; A61P0007-10 [I]
IPCR A61K0039-395 [I]; A61P0007-02 [I]; A61P0007-10 [I]; A61P0009-00 [I]; A61P0009-04 [I]; A61P0009-06 [I]; A61P0009-10 [I]; A61P0009-12 [I]; A61P0039-06 [I]; C12N0005-071 [I]; C12N0015-113 [I]

L2 ANSWER 208 OF 256 USPAT2 on STN
Full Text
AN 2011:237523 USPAT2
TI Cycloundecadepsipeptide compounds and use of said compounds as a medicament
IN Wenger, Roland, Riehen, SWITZERLAND
Mutter, Manfred, Belmont-Sur-Lausanne, SWITZERLAND
Garrouste, Patrick, Saxon, SWITZERLAND
Lysek, Robert, Martigny, SWITZERLAND
Turpin, Olivier, Martigny, SWITZERLAND
Vuagniaux, Gregoire, Lausanne, SWITZERLAND
Nicolas, Valerie, St-Prex, SWITZERLAND
Novaroli Zanolari, Laura, Lausanne, SWITZERLAND
Crabbe, Rafael, Bursins, SWITZERLAND
PA Debio Recherche Pharmaceutique S.A., Martigny, SWITZERLAND (non-U.S. corporation)
PI US 8501683 B2 20130806
WO 2010052559 20100514
AI US 2011-998588 20091106 (12)
WO 2009-IB7361 20091106
20110505 PCT 371 date
PRAI WO 2008-IB2982 20081106
DT Utility
FS GRANTED
LN.CNT 1819
INCL INCLM: 514/004.300
INCLS: 514/021.100
NCL NCLM: 514/004.300; 424/085.700
NCLS: 514/021.100; 424/094.500; 530/317.000
CPC CPCI C07K0011-02 [I]; A61K0038-212 [I]
CPCI-2 C07K0011-02 [I]; A61K0038-212 [I]
IPC IPCI A61K0038-21 [I]; C07K0011-02 [I]; A61K0038-15 [I]; A61K0038-45 [I]; A61P0031-14 [I]
IPCI-2 A61K0038-12 [I]
IPCR A61K0038-21 [I]; A61K0038-15 [I]; A61K0038-45 [I]; A61P0031-14 [I]; C07K0011-02 [I]

L2 ANSWER 209 OF 256 USPAT2 on STN
Full Text
AN 2011:91679 USPAT2
TI Selective inhibitors of histone deacetylase
IN Verner, Erik, Belmont, CA, UNITED STATES
Balasubramanian, Sriram, San Carlos, CA, UNITED STATES
Buggy, Joseph J., Mountain View, CA, UNITED STATES
PA Pharmacyclics, Inc., Sunnyvale, CA, UNITED STATES (U.S. corporation)
PI US 8466193 B2 20130618
WO 2009129335 20091022
AI US 2010-988271 20090415 (12)
WO 2009-US40709 20090415
20101201 PCT 371 date
PRAI US 2008-45198P 20080415 (61)
DT Utility
FS GRANTED
LN.CNT 7428
INCL INCLM: 514/427.000
INCLS: 548/561.000
NCL NCLM: 514/427.000; 424/450.000
NCLS: 548/561.000; 424/085.200; 424/133.100; 514/019.300; 514/043.000; 514/049.000; 514/114.000; 514/235.500; 514/383.000; 544/141.000
CPC CPCI C07D0207-337 [I]; C07D0209-08 [I]; C07D0235-06 [I]; C07D0307-79 [I]; C07D0307-80 [I]; C07D0333-54 [I]; C07D0333-56 [I]; C07D0401-06 [I]; C07D0401-12 [I]; C07D0405-06 [I]; C07D0409-06 [I]; C07D0471-04 [I]

CPCI-2 C07D0207-337 [I]; C07D0209-08 [I]; C07D0235-06 [I]; C07D0307-79 [I]; C07D0307-80 [I]; C07D0333-54 [I]; C07D0333-56 [I]; C07D0401-06 [I]; C07D0401-12 [I]; C07D0405-06 [I]; C07D0409-06 [I]; C07D0471-04 [I]
IPC IPCI A61K0009-127 [I]; C07D0207-337 [I]; C07D0413-12 [I]; A61K0031-40 [I]; A61K0031-5377 [I]; A61K0038-08 [I]; A61K0038-20 [I]; A61K0039-395 [I]; A61K0031-661 [I]; A61K0031-4196 [I]; A61K0031-706 [I]; A61K0031-7068 [I]; A61P0019-02 [I]; A61P0019-06 [I]; A61P0017-06 [I]; A61P0029-00 [I]; A61P0011-06 [I]; A61P0007-06 [I]; A61P0011-00 [I]
IPCI-2 A01N0043-36 [I]
IPCR A61K0009-127 [I]; A61K0031-40 [I]; A61K0031-4196 [I]; A61K0031-5377 [I]; A61K0031-661 [I]; A61K0031-706 [I]; A61K0031-7068 [I]; A61K0038-08 [I]; A61K0038-20 [I]; A61K0039-395 [I]; A61P0007-06 [I]; A61P0011-00 [I]; A61P0011-06 [I]; A61P0017-06 [I]; A61P0019-02 [I]; A61P0019-06 [I]; A61P0029-00 [I]; C07D0207-337 [I]; C07D0413-12 [I]
EXF 548/561; 514/427

L2 ANSWER 210 OF 256 USPAT2 on STN
Full Text
AN 2010:348825 USPAT2
TI Treatment of inflammatory diseases
IN Soliven, Betty C., Chicago, IL, UNITED STATES
PA The University of Chicago, Chicago, IL, UNITED STATES (U.S. corporation)
PI US 8258150 B2 20120904
WO 2008021532 20080221
AI US 2010-377736 20070817 (12)
WO 2007-US18331 20070817
20100825 PCT 371 date
PRAI US 2006-838222P 20060817 (60)
DT Utility
FS GRANTED
LN.CNT 1138
INCL INCLM: 514/291.000
INCLS: 424/283.100
NCL NCLM: 514/291.000; 424/130.100
NCLS: 424/283.100; 514/017.700; 514/090.000; 514/114.000; 514/171.000; 514/364.000; 514/653.000
CPC CPCI A61K0031-133 [I]; A61K0031-138 [I]; A61K0031-4245 [I]; A61K0031-56 [I]; A61K0031-661 [I]; A61K0045-06 [I]
CPCI-2 A61K0031-133 [I]; A61K0031-138 [I]; A61K0031-4245 [I]; A61K0031-56 [I]; A61K0031-661 [I]; A61K0045-06 [I]
IPC IPCI A61K0031-137 [I]; A61K0031-661 [I]; A61K0031-4245 [I]; A61K0039-395 [I]; A61K0031-56 [I]; A61K0038-13 [I]; A61K0031-675 [I]; A61P0025-02 [I]; A61P0037-02 [I]
IPCI-2 A61K0031-137 [I]
IPCR A61K0031-137 [I]

L2 ANSWER 211 OF 256 USPAT2 on STN
Full Text
AN 2010:342740 USPAT2
TI Compounds that modulate intracellular calcium
IN Velicelebi, Gonul, San Diego, CA, UNITED STATES
Stauderman, Kenneth A., San Diego, CA, UNITED STATES
King, Frank, Bishops Stortford, UNITED KINGDOM
Pei, Yazhong, San Diego, CA, UNITED STATES
Whitten, Jeffrey P., Santee, CA, UNITED STATES
PA Calcimedica, Inc., La Jolla, CA, UNITED STATES (U.S. corporation)
PI US 8389567 B2 20130305
WO 2009076454 20090618
AI US 2010-747604 20081210 (12)
WO 2008-US86254 20081210
20100817 PCT 371 date
PRAI US 2007-13227P 20071212 (61)
DT Utility
FS GRANTED
LN.CNT 6878
INCL INCLM: 514/447.000
NCL NCLM: 514/447.000
NCLS: 544/333.000; 546/256.000; 546/272.100; 546/275.400; 546/279.100; 548/245.000; 548/372.100; 548/532.000; 549/069.000

CPC CPCI C07D0403-04 [I]; A61K0031-381 [I]; A61K0031-415 [I]; A61K0031-4436 [I]; A61K0031-4439 [I]; A61K0031-444 [I]; A61K0031-5025 [I]; A61K0031-506 [I]; A61K0045-06 [I]; C07D0207-456 [I]; C07D0213-55 [I]; C07D0213-80 [I]; C07D0231-38 [I]; C07D0231-40 [I]; C07D0261-18 [I]; C07D0333-38 [I]; C07D0333-68 [I]; C07D0333-74 [I]; C07D0401-04 [I]; C07D0401-12 [I]; C07D0401-14 [I]; C07D0409-12 [I]; C07D0413-04 [I]; C07D0413-14 [I]; C07D0495-04 [I]
CPCI-2 C07D0403-04 [I]; A61K0031-381 [I]; A61K0031-415 [I]; A61K0031-4436 [I]; A61K0031-4439 [I]; A61K0031-444 [I]; A61K0031-5025 [I]; A61K0031-506 [I]; A61K0045-06 [I]; C07D0207-456 [I]; C07D0213-55 [I]; C07D0213-80 [I]; C07D0231-38 [I]; C07D0231-40 [I]; C07D0261-18 [I]; C07D0333-38 [I]; C07D0333-68 [I]; C07D0333-74 [I]; C07D0401-04 [I]; C07D0401-12 [I]; C07D0401-14 [I]; C07D0409-12 [I]; C07D0413-04 [I]; C07D0413-14 [I]; C07D0495-04 [I]
IPC IPCI A61K0031-381 [I]; C07D0261-18 [I]; C07D0413-14 [I]; C07D0231-38 [I]; C07D0413-04 [I]; C07D0401-04 [I]; C07D0403-04 [I]; C07D0401-14 [I]; C07D0207-50 [I]; C07D0333-36 [I]; A61P0029-00 [I]; A61P0013-12 [I]; A61P0001-16 [I]; A61P0011-00 [I]; A61P0001-00 [I]; A61P0017-00 [I]; A61P0019-10 [I]; A61P0003-10 [I]; A61P0007-06 [I]; A61P0011-02 [I]; A61P0037-08 [I]; A61P0037-02 [I]; A61P0027-02 [I]; A61P0017-06 [I]; A61P0025-00 [I]
IPCI-2 A61K0031-381 [I]
IPCR A61K0031-381 [I]
EXF 514/447; 549/69

L2 ANSWER 212 OF 256 USPAT2 on STN
Full Text
AN 2010:188549 USPAT2
TI Heterocyclic substituted pyridine compounds with CXCR3 antagonist activity
IN Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Kozlowski, Joseph A., Princeton, NJ, UNITED STATES
Shih, Neng-Yang, Lexington, MA, UNITED STATES
McGuinness, Brian F., Plainsboro, NJ, UNITED STATES
Hobbs, Douglas W., Chesterfield, MS, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
Pharmacopeia, Inc., Princeton, NJ, UNITED STATES (U.S. corporation)
PI US 8017616 B2 20110913
AI US 2009-642931 20091221 (12)
RLI Continuation of Ser. No. US 2007-688014, filed on 19 Mar 2007, Pat. No. US 7786124
PRAI US 2006-784504P 20060321 (60)
DT Utility
FS GRANTED
LN.CNT 3250
INCL INCLM: 514/253.090
INCLS: 514/253.100; 544/364.000
NCL NCLM: 514/253.090; 514/253.100
NCLS: 514/253.100; 544/364.000
CPC CPCI C07D0401-14 [I]; C07D0413-14 [I]
CPCI-2 C07D0401-14 [I]; C07D0413-14 [I]
IPC IPCI A61K0031-496 [I]; C07D0413-14 [I]; C07D0401-14 [I]; C07D0417-14 [I]; A61P0019-02 [I]; A61P0017-06 [I]; A61P0011-00 [I]
IPCI-2 A61K0031-496 [I]; C07D0413-14 [I]
IPCR A61K0031-496 [I]; C07D0413-14 [I]

L2 ANSWER 213 OF 256 USPAT2 on STN
Full Text
AN 2010:76378 USPAT2
TI Methods for modulating inflammatory and/or immune responses
IN Marshall, Vivienne S., Glenshaw, PA, UNITED STATES
Banas, Richard A., Turtle Creek, PA, UNITED STATES
Trumpower, Catherine J., Pittsburgh, PA, UNITED STATES
PI US 8221741 B2 20120717
WO 2008088738 20080724
AI US 2009-448903 20080111 (12)
WO 2008-US396 20080111
20090713 PCT 371 date
PRAI US 2007-880745P 20070117 (60)

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       US 2007-902440P         20070221 (60)
       US 2007-997604P         20071004 (60)
DT     Utility
FS     GRANTED
LN.CNT 1978
INCL   INCLM: 424/093.210
       INCLS: 424/093.700; 424/184.100; 424/141.100; 424/173.100
NCL    NCLM:  424/093.210; 424/085.400
       NCLS:  424/093.700; 424/141.100; 424/173.100; 424/184.100
CPC    CPCI   C12N0005-0636 [I]; C12N0005-0605 [I]; C12N2501-23; C12N2502-02
       CPCI-2 C12N0005-0636 [I]; C12N0005-0605 [I]; C12N2501-23; C12N2502-02
IPC    IPCI   A61K0039-395 [I]; A61K0035-12 [I]; A61K0038-21 [I]; A61P0037-06
              [I]; A61P0029-00 [I]
       IPCI-2 A61K0039-00 [I]; A61K0035-12 [I]; A61K0038-21 [I]
       IPCR   A61K0039-00 [I]; A61K0035-12 [I]; A61K0038-21 [I]; C12N0005-073
              [I]; C12N0005-0783 [I]

L2   ANSWER 214 OF 256  USPAT2 on STN
Full Text
AN     2009:362673  USPAT2
TI     Fc receptor binding proteins
IN     TenHoor, Christopher, Hopkinton, MA, UNITED STATES
       Muruganandam, Arumugam, Bangalore, INDIA
       Ladner, Robert Charles, Ijamsville, MD, UNITED STATES
       Wood, Clive, Boston, MA, UNITED STATES
       Bitonti, Alan J., Acton, MA, UNITED STATES
       Stattel, James, Hagerstown, MD, UNITED STATES
       McDonnell, Kevin, Waltham, MA, UNITED STATES
       Liu, Liming, Upper Dublin, PA, UNITED STATES
       Dumont, Jennifer, Groton, MA, UNITED STATES
       Sato, Aaron, Richmond, CA, UNITED STATES
PA     Dyax Corp., Burlington, MA, UNITED STATES (U.S. corporation)
       Syntonix Pharmaceuticals, Inc., Waltham, MA, UNITED STATES (U.S.
       corporation)
PI     US 8273351          B2  20120925
AI     US 2009-429529          20090424 (12)
PRAI   US 2008-48152P          20080425 (61)
       US 2008-48500P          20080428 (61)
DT     Utility
FS     GRANTED
LN.CNT 6646
INCL   INCLM: 424/133.100
       INCLS: 530/389.100
NCL    NCLM:  424/133.100; 424/172.100
       NCLS:  530/389.100; 436/501.000; 536/023.530
CPC    CPCI   C07K0016-283 [I]; A61K2039-505; C07K2317-21; C07K2317-56;
              C07K2317-565; G01N0033-68 [I]
       CPCI-2 C07K0016-283 [I]; A61K2039-505; C07K2317-21; C07K2317-56;
              C07K2317-565; G01N0033-68 [I]
IPC    IPCI   A61K0039-395 [I]; C07K0016-00 [I]; C07H0021-00 [I]; G01N0033-53
              [I]; A61P0037-00 [I]
       IPCI-2 A61K0039-395 [I]; C07K0016-00 [I]; C07H0021-00 [N]; G01N0033-53
              [N]
       IPCR   A61K0039-395 [I]; C07H0021-00 [N]; C07K0016-00 [I]; G01N0033-53
              [N]
EXF    424/133.1; 530/389.1

L2   ANSWER 215 OF 256  USPAT2 on STN
Full Text
AN     2009:225737  USPAT2
TI     Immunomodulation by altering sphingosine 1-phosphate lyase (SPL)
       activity
IN     Saba, Julie D., Oakland, CA, UNITED STATES
PA     Children's Hospital & Research Center at Oakland, Oakland, CA, UNITED
       STATES (U.S. corporation)
PI     US 8124111          B2  20120228
       WO 2006135862           20061221
AI     US 2008-917029          20060608 (11)
       WO 2006-US22805         20060608
                               20080908  PCT 371 date
PRAI   US 2005-689611P         20050610 (60)
DT     Utility
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FS GRANTED
LN.CNT 2310
INCL INCLM: 424/278.100
INCLS: 514/009.000
NCL NCLM: 424/278.100; 424/139.100
NCLS: 514/021.200; 424/094.500; 514/345.000; 514/558.000
CPC CPCI C12N0009-88 [I]; A01K2267-03; C12N2799-022
CPCI-2 C12N0009-88 [I]; A01K2267-03; C12N2799-022
IPC IPCI A61K0039-395 [I]; A61K0031-7105 [I]; A61K0031-4415 [I]; A61K0031-164 [I]; A61K0031-711 [I]; A61K0038-45 [I]; A61P0037-00 [I]
IPCI-2 A61K0039-00 [I]; C12N0009-88 [N]
IPCR A61K0039-00 [I]; C12N0009-88 [N]

L2 ANSWER 216 OF 256 USPAT2 on STN
Full Text
AN 2009:219334 USPAT2
TI Oligodendrocyte precursor cell composition and methods of use
IN Bieberich, Erhard, Augusta, GA, UNITED STATES
PA Georgia Health Sciences University, Augusta, GA, UNITED STATES (U.S. corporation)
PI US 7985586 B2 20110726
AI US 2009-365381 20090204 (12)
PRAI US 2008-63315P 20080204 (61)
DT Utility
FS GRANTED
LN.CNT 2359
INCL INCLM: 435/366.000
INCLS: 435/325.000; 435/374.000
NCL NCLM: 435/366.000; 424/093.700
NCLS: 435/325.000; 435/374.000; 435/368.000; 435/378.000
CPC CPCI C12N0005-0622 [I]; A61K0031-164 [I]; A61K0035-30 [I]; C12N0005-0081 [I]; C12N0005-0623 [I]; C12N2500-36; C12N2501-48; C12N2506-02
CPCI-2 C12N0005-0622 [I]; A61K0031-164 [I]; A61K0035-30 [I]; C12N0005-0081 [I]; C12N0005-0623 [I]; C12N2500-36; C12N2501-48; C12N2506-02
IPC IPCI A61K0035-30 [I]; C12N0005-08 [I]; A61P0025-28 [I]; C12N0005-02 [I]
IPCI-2 C12N0005-00 [I]
IPCR C12N0005-00 [I]; C12N0005-079 [I]; C12N0005-0797 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 217 OF 256 USPAT2 on STN
Full Text
AN 2009:173940 USPAT2
TI Aminopropanol derivatives
IN Albert, Rainer, Basel, SWITZERLAND
Francotte, Eric, Nuglar, SWITZERLAND
Zecri, Frederic, Bartenheim, FRANCE
Zollinger, Markus, Mohlin, SWITZERLAND
PA Novartis AG, Basel, SWITZERLAND (non-U.S. corporation)
PI US 7902175 B2 20110308
AI US 2009-391298 20090224 (12)
RLI Division of Ser. No. US 1900-569696, Pat. No. US 7528120 A 371 of International Ser. No. WO 2004-EP9589, filed on 27 Aug 2004
PRAI GB 2003-20196 20030828
GB 2003-24206 20031015
DT Utility
FS GRANTED
LN.CNT 1168
INCL INCLM: 514/080.000
INCLS: 514/114.000; 548/414.000; 564/015.000
NCL NCLM: 514/080.000
NCLS: 514/114.000; 548/414.000; 564/015.000
CPC CPCI C07F0009-5728 [I]; C07D0209-46 [I]; C07D0263-14 [I]; C07F0009-094 [I]; C07F0009-653 [I]
CPCI-2 C07F0009-5728 [I]; C07D0209-46 [I]; C07D0263-14 [I]; C07F0009-094 [I]; C07F0009-653 [I]
IPC IPCI A61K0031-675 [I]; C07F0009-547 [I]; C07F0009-06 [I]; A61K0031-66 [I]
IPCI-2 A61K0031-675 [I]; A61K0031-661 [I]; C07F0009-572 [I]; C07F0009-06

[I]
IPCR A61K0031-675 [I]; A61K0031-661 [I]; A61P0037-02 [I]; C07D0209-46 [I]; C07D0263-14 [I]; C07F0009-06 [I]; C07F0009-09 [I]; C07F0009-572 [I]; C07F0009-653 [I]
EXF 514/114; 564/15
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 218 OF 256 USPAT2 on STN
Full Text
AN 2009:152815 USPAT2
TI Compounds that modulate intracellular calcium
IN Velicelebi, Gonul, San Diego, CA, UNITED STATES
Stauderman, Kenneth A., San Diego, CA, UNITED STATES
Pleynet, David P. M., San Diego, CA, UNITED STATES
Cheng, Soan, San Diego, CA, UNITED STATES
Whitten, Jeffrey P., Santee, CA, UNITED STATES
PA Calcimedica, Inc., La Jolla, CA, UNITED STATES (U.S. corporation)
PI US 8263641 B2 20120911
AI US 2008-192812 20080815 (12)
PRAI US 2007-971161P 20070910 (60)
DT Utility
FS GRANTED
LN.CNT 6562
INCL INCLM: 514/447.000
INCLS: 549/069.000
NCL NCLM: 514/447.000; 514/443.000
NCLS: 549/069.000; 435/375.000; 549/057.000
CPC CPCI C07D0333-68 [I]; A61K0031-00 [I]; A61K0031-405 [I]
CPCI-2 C07D0333-68 [I]; A61K0031-00 [I]; A61K0031-405 [I]
IPC IPCI A61K0031-381 [I]; C12N0005-06 [I]; C07D0409-12 [I]; C07D0333-36 [I]
IPCI-2 A61K0031-381 [I]; C07D0333-36 [I]
IPCR A61K0031-381 [I]; C07D0333-36 [I]
EXF 549/69; 514/447

L2 ANSWER 219 OF 256 USPAT2 on STN
Full Text
AN 2009:32548 USPAT2
TI Use of S1P receptor agonists in heart diseases
IN Brinkmann, Volker, Freiburg, GERMANY, FEDERAL REPUBLIC OF
Feutren, Gilles, Mulhouse, FRANCE
Hof, Robert Paul, Gelterkinden, SWITZERLAND
PA Novartis AG, Basel, SWITZERLAND (non-U.S. corporation)
PI US 7910626 B2 20110322
AI US 2008-244422 20081002 (12)
RLI Continuation of Ser. No. US 1900-521297, ABANDONED A 371 of International Ser. No. WO 2003-EP8085, filed on 23 Jul 2003
PRAI GB 2002-17152 20020724
DT Utility
FS GRANTED
LN.CNT 517
INCL INCLM: 514/646.000
INCLS: 514/653.000; 558/169.000
NCL NCLM: 514/646.000; 514/020.100
NCLS: 514/653.000; 558/169.000; 514/114.000; 514/357.000; 514/408.000; 514/649.000; 514/651.000
CPC CPCI A61K0045-06 [I]; A61K0031-13 [I]; A61K0031-137 [I]; A61K0038-2242 [I]; A61K0038-556 [I]
CPCI-2 A61K0045-06 [I]; A61K0031-13 [I]; A61K0031-137 [I]; A61K0038-2242 [I]; A61K0038-556 [I]
IPC IPCI A61K0038-17 [I]; A61K0031-66 [I]; A61K0031-137 [I]; A61P0009-04 [I]; A61P0009-10 [I]; A61P0009-06 [I]; A61K0031-44 [I]; A61K0031-40 [I]
IPCI-2 A01N0033-02 [I]; A61K0031-135 [I]
IPCR A01N0033-02 [I]; C07D0333-56 [I]; A61K0031-13 [I]; A61K0031-135 [I]; A61K0031-137 [I]; A61K0031-381 [I]; A61K0031-661 [I]; A61K0038-22 [I]; A61K0038-55 [I]; A61K0045-00 [I]; A61K0045-06 [I]; A61P0009-00 [I]; A61P0009-04 [I]; A61P0009-06 [I]; A61P0009-10 [I]; A61P0043-00 [I]
EXF 514/183
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 220 OF 256 USPAT2 on STN
Full Text
AN 2008:333414 USPAT2
TI Heterocyclic substituted pyridine or phenyl compounds with CXCR3 antagonist activity
IN Anilkumar, Gopinadhan N., Edison, NJ, UNITED STATES
Zeng, Qingbei, Edison, NJ, UNITED STATES
Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Kozlowski, Joseph A., Princeton, NJ, UNITED STATES
McGuinness, Brian F., Plainsboro, NJ, UNITED STATES
Hobbs, Douglas W., Chesterfield, MO, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
Pharmacopeia Drug Discovery, Inc., Princeton, NJ, UNITED STATES (U.S. corporation)
PI US 7799789 B2 20100921
AI US 2008-147140 20080626 (12)
RLI Division of Ser. No. US 2006-353609, filed on 14 Feb 2006, Pat. No. US 7417045
PRAI US 2005-653332P 20050216 (60)
DT Utility
FS GRANTED
LN.CNT 3391
INCL INCLM: 514/253.010
INCLS: 514/253.100; 544/360.000
NCL NCLM: 514/253.010; 424/085.600
NCLS: 514/253.100; 544/360.000; 424/133.100; 514/001.100; 514/002.400; 514/161.000; 514/252.180; 514/253.090; 544/295.000; 544/364.000
CPC CPCI C07D0417-14 [I]; C07D0401-04 [I]; C07D0401-14 [I]; C07D0413-14 [I]
CPCI-2 C07D0417-14 [I]; C07D0401-04 [I]; C07D0401-14 [I]; C07D0413-14 [I]
IPC IPCI A61K0038-21 [I]; C07D0401-14 [I]; A61K0031-496 [I]; A61K0038-13 [I]; A61K0031-573 [I]; A61P0029-00 [I]; A61K0031-505 [I]; A61K0031-606 [I]
IPCI-2 A61K0031-497 [I]; C07D0401-00 [I]
IPCR A61K0031-497 [I]; C07D0401-00 [I]
EXF 514/253.1; 514/253.09; 544/360
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 221 OF 256 USPAT2 on STN
Full Text
AN 2008:327426 USPAT2
TI Use of azapaullones for preventing and treating pancreatic autoimmune disorders
IN Mussmann, Rainer, Gottingen, GERMANY, FEDERAL REPUBLIC OF
Kunick, Conrad Peter, Hamburg, GERMANY, FEDERAL REPUBLIC OF
Stukenbrock, Hendrik, Braunschweig, GERMANY, FEDERAL REPUBLIC OF
Geese, Marcus, Gottingen, GERMANY, FEDERAL REPUBLIC OF
Kegel, Simone, Uslar, GERMANY, FEDERAL REPUBLIC OF
Burk, Ulrike, Gottingen, GERMANY, FEDERAL REPUBLIC OF
PA Develogen Atkiengesellschaft, Goettingen, GERMANY, FEDERAL REPUBLIC OF (non-U.S. corporation)
Technische Universitaet Carolo-Wilhelmina Zu Braunschweig, Braunschweig, GERMANY, FEDERAL REPUBLIC OF (non-U.S. corporation)
PI US 7968535 B2 20110628
WO 2006117221 20061109
AI US 2008-913486 20060504 (11)
WO 2006-EP4186 20060504
20080603 PCT 371 date
PRAI EP 2005-9846 20050504
EP 2005-15986 20050722
EP 2005-23168 20051024
EP 2006-1327 20060123
DT Utility
FS GRANTED
LN.CNT 1719
INCL INCLM: 514/212.060
INCLS: 540/521.000
NCL NCLM: 514/212.060; 514/215.000
NCLS: 540/521.000
CPC CPCI C07D0471-14 [I]; A61K0031-55 [I]; A61K0045-00 [I]; A61K0045-06 [I]

CPCI-2 C07D0471-14 [I]; A61K0031-55 [I]; A61K0045-00 [I]; A61K0045-06 [I]
IPC IPCI A61K0031-55 [I]; C07D0487-14 [I]; A61P0005-48 [I]; A61P0001-18 [I]; A61P0003-10 [I]; A61P0025-28 [I]
IPCI-2 C07D0471-14 [I]; A61K0031-55 [I]
IPCR C07D0471-14 [I]; A61K0031-55 [I]
EXF 540/521; 514/212.06
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 222 OF 256 USPAT2 on STN
Full Text
AN 2008:66423 USPAT2
TI Heterocyclic substituted piperazine compounds with CXCR3 antagonist activity
IN Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Kozlowski, Joseph A., Princeton, NJ, UNITED STATES
Shih, Neng-Yang, Warren, NJ, UNITED STATES
McGuinness, Brian F., Plainsboro, NJ, UNITED STATES
Hobbs, Douglas W., Chesterfield, MO, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
Pharmacopeia, Inc., Princeton, NJ, UNITED STATES (U.S. corporation)
PI US 7902199 B2 20110308
AI US 2007-776901 20070712 (11)
PRAI US 2006-831053P 20060714 (60)
DT Utility
FS GRANTED
LN.CNT 2698
INCL INCLM: 514/253.130
INCLS: 544/364.000
NCL NCLM: 514/253.130; 514/253.010
NCLS: 544/364.000; 544/360.000
CPC CPCI C07D0401-14 [I]
CPCI-2 C07D0401-14 [I]
IPC IPCI A61K0031-497 [I]; A61P0025-00 [I]; C07D0401-02 [I]
IPCI-2 A61K0031-496 [I]; C07D0401-12 [I]; C07D0401-14 [I]
IPCR A61K0031-496 [I]; C07D0401-12 [I]; C07D0401-14 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 223 OF 256 USPAT2 on STN
Full Text
AN 2008:44907 USPAT2
TI Methods of inhibiting vascular permeability and apoptosis
IN Hla, Timothy, Avon, CT, UNITED STATES
Sanchez, Teresa, West Hartford, CT, UNITED STATES
Claffey, Kevin Patrick, Burlington, CT, UNITED STATES
Paik, Ji-Hye, New Britain, CT, UNITED STATES
PA University of Connecticut, Farmington, CT, UNITED STATES (U.S. corporation)
PI US 7838562 B2 20101123
WO 2005002559 20050113
AI US 2007-562305 20040618 (10)
WO 2004-US19420 20040618
20070309 PCT 371 date
DT Utility
FS GRANTED
LN.CNT 1036
INCL INCLM: 514/646.000
INCLS: 514/653.000; 558/169.000
NCL NCLM: 514/646.000; 514/667.000
NCLS: 514/653.000; 558/169.000
CPC CPCI A61K0031-133 [I]; A61K0031-00 [I]
CPCI-2 A61K0031-133 [I]; A61K0031-00 [I]
IPC IPCI A61K0031-131 [I]; A61P0007-04 [I]; A61P0009-10 [I]
IPCI-2 A01N0033-02 [I]; A61K0031-135 [I]
IPCR A01N0033-02 [I]; A61K0031-00 [I]; A61K0031-133 [I]; A61K0031-135 [I]; A61P0009-00 [I]
EXF 514/183
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 224 OF 256 USPAT2 on STN
Full Text
AN 2008:44851 USPAT2

TI Heterocyclic substituted pyridine compounds with CXCR3 antagonist activity
IN Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Kozlowski, Joseph A., Princeton, NJ, UNITED STATES
Shih, Neng-Yang, Warren, NJ, UNITED STATES
McGuinness, Brian F., Plainsboro, NJ, UNITED STATES
Hobbs, Douglas W., Yardley, PA, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
PI US 7786124 B2 20100831
AI US 2007-688014 20070319 (11)
PRAI US 2006-784504P 20060321 (60)
DT Utility
FS GRANTED
LN.CNT 3164
INCL INCLM: 514/253.090
INCLS: 514/253.100; 544/364.000
NCL NCLM: 514/253.090; 514/253.010
NCLS: 514/253.100; 544/364.000; 544/360.000
CPC CPCI C07D0401-14 [I]; C07D0413-14 [I]
CPCI-2 C07D0401-14 [I]; C07D0413-14 [I]
IPC IPCI A61K0031-497 [I]; A61P0029-00 [I]; C07D0401-02 [I]
IPCI-2 C07D0413-14 [I]; A61K0031-496 [I]
IPCR C07D0413-14 [I]; A61K0031-496 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 225 OF 256 USPAT2 on STN
Full Text
AN 2007:322602 USPAT2
TI Indole derivatives as inhibitors of histone deacetylase
IN Buggy, Joseph J., Mountain View, CA, UNITED STATES
Balasubramanian, Sriram, San Carlos, CA, UNITED STATES
Verner, Erik, San Mateo, CA, UNITED STATES
Tai, Vincent W. F., San Mateo, CA, UNITED STATES
Lee, Chang-Sun, Belle Mead, NJ, UNITED STATES
PA Pharmacylics, Inc., Sunnyvale, CA, UNITED STATES (U.S. corporation)
PI US 8338416 B2 20121225
AI US 2007-687565 20070316 (11)
PRAI US 2006-783287P 20060316 (60)
DT Utility
FS GRANTED
LN.CNT 7187
INCL INCLM: 514/235.200
INCLS: 514/320.000; 514/415.000; 544/143.000; 546/201.000; 548/452.000
NCL NCLM: 514/235.200
NCLS: 514/320.000; 514/415.000; 544/143.000; 546/201.000; 548/452.000
CPC CPCI A61K0031-405 [I]; A61K0031-55 [I]; A61K0045-06 [I]; C07D0209-08 [I]; C07D0209-12 [I]; C07D0209-14 [I]
CPCI-2 A61K0031-405 [I]; A61K0031-55 [I]; A61K0045-06 [I]; C07D0209-08 [I]; C07D0209-12 [I]; C07D0209-14 [I]
IPC IPCI C07D0209-04 [I]; A61K0031-404 [I]; A61K0031-4523 [I]; A61K0031-5377 [I]; C07D0413-02 [I]; C07D0401-02 [I]; A61P0035-00 [I]; A61P0035-02 [I]
IPCI-2 C07D0401-02 [I]; C07D0209-04 [I]; C07D0413-02 [I]; A61K0031-404 [I]; A61K0031-4523 [I]; A61K0031-5377 [I]; A61P0035-00 [I]; A61P0035-02 [I]
IPCR C07D0401-02 [I]; A61K0031-404 [I]; A61K0031-4523 [I]; A61K0031-5377 [I]; A61P0035-00 [I]; A61P0035-02 [I]; C07D0209-04 [I]; C07D0413-02 [I]

L2 ANSWER 226 OF 256 USPAT2 on STN
Full Text
AN 2007:191244 USPAT2
TI Compound capable of binding S1P receptor and pharmaceutical use thereof
IN Nakade, Shinji, Tsukuba, JAPAN
Mizuno, Hirotaka, Tsukuba, JAPAN
Ono, Takeji, Tsukuba, JAPAN
Minami, Masashi, Tsukuba, JAPAN
Saga, Hiroshi, Tsukuba, JAPAN
Hagiya, Hiroshi, Tsukuba, JAPAN
Komiya, Takaki, Tsukuba, JAPAN
Habashita, Hiromu, Mishima-gun, JAPAN
Kurata, Haruto, Mishima-gun, JAPAN

Ohtsuki, Kazuhiro, Mishima-gun, JAPAN
Kusumi, Kensuke, Mishima-gun, JAPAN
PA Ono Pharmaceutical Co., Ltd., Osaka, JAPAN (non-U.S. corporation)
PI US 7825109 B2 20101102
WO 2005020882 20050310
AI US 2006-569831 20040827 (10)
WO 2004-JP12768 20040827
20060228 PCT 371 date
PRAI JP 2003-306088 20030829
JP 2004-110573 20040402
JP 2004-169958 20040608
JP 2004-198523 20040705
DT Utility
FS GRANTED
LN.CNT 7161
INCL INCLM: 514/210.170
INCLS: 548/953.000
NCL NCLM: 514/210.170
NCLS: 548/953.000; 514/319.000; 514/419.000; 514/567.000; 546/205.000; 548/495.000; 562/444.000
CPC CPCI C07D0205-04 [I]; C07C0229-14 [I]; C07C0229-22 [I]; C07C0229-36 [I]; C07C0233-47 [I]; C07C0255-16 [I]; C07C0255-41 [I]; C07C0271-22 [I]; C07C0323-12 [I]; C07C2101-08; C07C2101-14; C07D0211-70 [I]; C07D0217-04 [I]
CPCI-2 C07D0205-04 [I]; C07C0229-14 [I]; C07C0229-22 [I]; C07C0229-36 [I]; C07C0233-47 [I]; C07C0255-16 [I]; C07C0255-41 [I]; C07C0271-22 [I]; C07C0323-12 [I]; C07C2101-08; C07C2101-14; C07D0211-70 [I]; C07D0217-04 [I]
IPC IPCI A61K0031-445 [I]; A61K0031-405 [I]; A61K0031-197 [I]; A61K0031-397 [I]; A61K0031-198 [I]
IPCI-2 C07D0205-00 [I]; A61K0031-397 [I]
IPCR C07D0205-00 [I]; A61K [I]; A61K0031-11 [I]; A61K0031-135 [I]; A61K0031-197 [I]; A61K0031-198 [I]; A61K0031-222 [I]; A61K0031-27 [I]; A61K0031-277 [I]; A61K0031-397 [I]; A61K0031-472 [I]; A61K0045-00 [I]; A61P0037-06 [I]; A61P0037-08 [I]; A61P0043-00 [I]; C07C0229-14 [I]; C07C0229-22 [I]; C07C0229-36 [I]; C07C0233-47 [I]; C07C0255-16 [I]; C07C0255-41 [I]; C07C0271-22 [I]; C07D0205-04 [I]; C07D0211-04 [I]; C07D0217-04 [I]; C07D0405-06 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 227 OF 256 USPAT2 on STN
Full Text
AN 2007:95211 USPAT2
TI Substituted heterocyclic compounds with CXCR3 antagonist activity
IN Kim, Seong Heon, Livingston, NJ, UNITED STATES
Shankar, Bandarpalle B., Branchburg, NJ, UNITED STATES
Kozlowski, Joseph A., Princeton, NJ, UNITED STATES
Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Shih, Neng-Yang, Warren, NJ, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
PI US 7781437 B2 20100824
AI US 2006-545201 20061010 (11)
PRAI US 2005-725483P 20051011 (60)
DT Utility
FS GRANTED
LN.CNT 2367
INCL INCLM: 514/252.110
INCLS: 544/359.000; 544/405.000; 544/408.000; 546/207.000; 546/245.000; 548/131.000; 548/215.000; 548/250.000; 548/255.000; 548/311.100
NCL NCLM: 514/252.110
NCLS: 544/359.000; 544/405.000; 544/408.000; 546/207.000; 546/245.000; 548/131.000; 548/215.000; 548/250.000; 548/255.000; 548/311.100; 514/253.010; 544/357.000; 544/364.000
CPC CPCI C07D0401-14 [I]; C07D0401-08 [I]; C07D0413-14 [I]
CPCI-2 C07D0401-14 [I]; C07D0401-08 [I]; C07D0413-14 [I]
IPC IPCI A61K0031-497 [I]; A61K0031-496 [I]; C07D0403-14 [I]
IPCI-2 A61K0031-497 [I]
IPCR A61K0031-497 [I]
EXF 544/359
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 228 OF 256 USPAT2 on STN
Full Text
AN 2007:62774 USPAT2
TI Pyrazinyl substituted piperazine-piperidines with CXCR3 antagonist activity
IN Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Kim, Seong Heon, Livingston, NJ, UNITED STATES
Zeng, Qingbei, Edison, NJ, UNITED STATES
Wong, Michael K. C., North Brunswick, NJ, UNITED STATES
Anilkumar, Gopinadhan N., Edison, NJ, UNITED STATES
Jiang, Yueheng, Whitehouse Station, NJ, UNITED STATES
Yu, Wensheng, Edison, NJ, UNITED STATES
Kozlowski, Joseph A., Princeton, NJ, UNITED STATES
Shih, Neng-Yang, Warren, NJ, UNITED STATES
Shankar, Bandarpalle B., Branchburg, NJ, UNITED STATES
McGuinness, Brian F., Plainsboro, NJ, UNITED STATES
Dong, Guizhen, Dayton, NJ, UNITED STATES
Zawacki, Lisa Guise, Yardley, PA, UNITED STATES
Hobbs, Douglas W., Yardley, PA, UNITED STATES
Baldwin, John J., Gwynedd Valley, PA, UNITED STATES
Shao, Yuefei, Princeton, NJ, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
PI US 7868005 B2 20110111
AI US 2006-354138 20060214 (11)
PRAI US 2005-653338P 20050216 (60)
DT Utility
FS GRANTED
LN.CNT 3899
INCL INCLM: 514/252.110
INCLS: 544/357.000; 544/229.000; 514/063.000
NCL NCLM: 514/252.110
NCLS: 514/063.000; 544/229.000; 544/357.000
CPC CPCI C07D0405-14 [I]; C07D0401-12 [I]; C07D0401-14 [I]; C07D0413-14 [I]; C07D0417-14 [I]
CPCI-2 C07D0405-14 [I]; C07D0401-12 [I]; C07D0401-14 [I]; C07D0413-14 [I]; C07D0417-14 [I]
IPC IPCI A61K0031-497 [I]; C07D0403-14 [I]
IPCI-2 C07D0241-04 [I]; C07D0401-14 [I]; A61K0031-443 [I]; A61K0031-4433 [I]; A61K0031-4439 [I]; A61K0031-444 [I]; A61K0031-4436 [I]; A61K0031-695 [I]; C07F0007-18 [I]; A61P0025-28 [N]; A61P0035-00 [N]; A61P0029-00 [N]
IPCR C07D0241-04 [I]; A61K0031-443 [I]; A61K0031-4433 [I]; A61K0031-4436 [I]; A61K0031-4439 [I]; A61K0031-444 [I]; A61K0031-695 [I]; A61P0025-28 [N]; A61P0029-00 [N]; A61P0035-00 [N]; C07D0401-14 [I]; C07F0007-18 [I]
EXF 514/252.11; 544/357
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 229 OF 256 USPAT2 on STN
Full Text
AN 2007:36890 USPAT2
TI Peptides and peptide mimetics to treat pathologies characterized by an inflammatory response
IN Fogelman, Alan M., Beverly Hills, CA, UNITED STATES
Navab, Mohamad, Los Angeles, CA, UNITED STATES
Anantharamaiah, Gattadahalli M., Birmingham, AL, UNITED STATES
PA The Regents of the University of California, Oakland, CA, UNITED STATES (U.S. corporation)
The UAB Research Foundation, Birmingham, AL, UNITED STATES (U.S. corporation)
PI US 7723303 B2 20100525
AI US 2006-407390 20060418 (11)
RLI Continuation-in-part of Ser. No. US 2003-423830, filed on 25 Apr 2003, Pat. No. US 7199102, issued on 3 Apr 2007 Continuation-in-part of Ser. No. US 2002-273386, filed on 16 Oct 2002, Pat. No. US 7166578, issued on 23 Jan 2007 Continuation-in-part of Ser. No. US 2002-187215, filed on 28 Jun 2002, Pat. No. US 7144862, issued on 5 Dec 2006 Continuation-in-part of Ser. No. US 2001-896841, filed on 29 Jun 2001, Pat. No. US 6933279, issued on 23 Aug 2005 Continuation-in-part of Ser. No. US 2000-645454, filed on 24 Aug 2000, Pat. No. US 6664230, issued on 16 Dec 2003
PRAI US 2005-697495P 20050707 (60)
US 2005-676431P 20050429 (60)

DT Utility
FS GRANTED
LN.CNT 11426
INCL INCLM: 514/013.000
INCLS: 424/424.000; 530/326.000; 623/001.420
NCL NCLM: 435/006.160
NCLS: 424/424.000; 514/001.400; 514/001.500; 514/001.900; 514/002.100; 514/002.400; 514/003.200; 514/003.800; 514/007.300; 514/013.200; 514/015.400; 514/016.400; 514/016.800; 514/016.900; 514/017.800; 514/017.900; 514/018.200; 514/019.400; 530/326.000; 623/001.420
CPC CPCI A61L0031-16 [I]; A61K0038-00; A61L2300-25; A61L2300-40; C07K0007-08 [I]; C07K0014-775 [I]
CPCI-2 A61L0031-16 [I]; A61K0038-00; A61L2300-25; A61L2300-40; C07K0007-08 [I]; C07K0014-775 [I]
IPC IPCI A61K0038-10 [I]; C07K0007-08 [I]
IPCI-2 A61F0002-82 [I]; A61K0038-10 [I]; C07K0007-08 [I]
IPCR A61F0002-82 [I]; A61K0038-10 [I]; C07K0007-08 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 230 OF 256 USPAT2 on STN
Full Text
AN 2007:29810 USPAT2
TI Pharmaceutical co-crystal compositions
IN Almarsson, Orn, Shrewsbury, MA, UNITED STATES
Bourghol Hickey, Magali, Medford, MA, UNITED STATES
Peterson, Matthew L., Framingham, MA, UNITED STATES
Zaworotko, Michael J., Tampa, FL, UNITED STATES
Moulton, Brian, Providence, RI, UNITED STATES
Rodriguez-Hornedo, Nair, Ann Arbor, MI, UNITED STATES
PA University of South Florida, Tampa, FL, UNITED STATES (U.S. corporation)
The Regents of the University of Michigan, Ann Arbor, MI, UNITED STATES (U.S. corporation)
Transform Pharmaceuticals, Inc., Lexington, MA, UNITED STATES (U.S. corporation)
PI US 7927613 B2 20110419
AI US 2003-660202 20030911 (10)
RLI Continuation-in-part of Ser. No. WO 2003-US27772, filed on 4 Sep 2003, PENDING Continuation-in-part of Ser. No. US 2003-378956, filed on 1 Mar 2003, PENDING Continuation-in-part of Ser. No. US 1900-660022, PENDING Continuation-in-part of Ser. No. US 2003-637829, filed on 8 Aug 2003, ABANDONED Division of Ser. No. US 2002-295995, filed on 18 Nov 2002, Pat. No. US 6699840 Continuation of Ser. No. US 2002-232589, filed on 3 Sep 2002, Pat. No. US 6559293 Continuation of Ser. No. US 1900-660202, PENDING Continuation-in-part of Ser. No. US 2003-601092, filed on 20 Jun 2003, ABANDONED Continuation-in-part of Ser. No. US 2003-449307, filed on 30 May 2003, Pat. No. US 7078526
PRAI US 2002-360768P 20020301 (60)
US 2003-451213P 20030228 (60)
US 2003-463962P 20030418 (60)
US 2003-487064P 20030711 (60)
US 2002-406974P 20020830 (60)
US 2002-380288P 20020515 (60)
US 2002-356764P 20020215 (60)
US 2003-444315P 20030131 (60)
US 2003-439282P 20030110 (60)
US 2002-384152P 20020531 (60)
DT Utility
FS GRANTED
LN.CNT 23308
INCL INCLM: 424/400.000
INCLS: 514/217.000; 514/403.000
NCL NCLM: 424/400.000; 424/489.000
NCLS: 514/217.000; 514/403.000
CPC CPCI A61K0009-145 [I]; A61K0009-0004; A61K0009-1617 [I]; A61K0009-1652 [I]; A61K0009-2077 [I]; A61K0009-209; A61K0031-167 [I]; A61K0031-192 [I]; A61K0031-4166 [I]; A61K0031-55 [I]; A61K0031-616 [I]; A61K0047-10 [I]; A61K0047-32 [I]; C07C0051-412 [I]; C07C0051-43 [I]; C07C0233-25 [I]; C07D0223-26 [I]; C07D0233-74 [I]; C07D0495-04 [I]
CPCI-2 A61K0009-145 [I]; A61K0009-0004; A61K0009-1617 [I]; A61K0009-1652 [I]; A61K0009-2077 [I]; A61K0009-209; A61K0031-167 [I]; A61K0031-192 [I]; A61K0031-4166 [I]; A61K0031-55 [I];

A61K0031-616 [I]; A61K0047-10 [I]; A61K0047-32 [I]; C07C0051-412 [I]; C07C0051-43 [I]; C07C0233-25 [I]; C07D0223-26 [I]; C07D0233-74 [I]; C07D0495-04 [I]
IPC IPCI A61K0009-14 [I]
IPCI-2 A61K0009-00 [I]; A01N0043-46 [I]; A01N0043-56 [I]
IPCR A61K0009-00 [I]; A01N0043-46 [I]; A01N0043-56 [I]
EXF 424/400; 514/217; 514/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 231 OF 256 USPAT2 on STN
Full Text
AN 2007:24566 USPAT2
TI Pyridyl and phenyl substituted piperazine-piperidines with CXCR3 antagonist activity
IN McGuinness, Brian F., Plainsboro, NJ, UNITED STATES
Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Kozlowski, Joseph A., Princeton, NJ, UNITED STATES
Anilkumar, Gopinadhan N., Edison, NJ, UNITED STATES
Kim, Seong Heon, Livingston, NJ, UNITED STATES
Shih, Neng-Yang, Warren, NJ, UNITED STATES
Jenh, Chung-Her, Annandale, NJ, UNITED STATES
Zavodny, Paul J., Mountainside, NJ, UNITED STATES
Hobbs, Douglas W., Yardley, PA, UNITED STATES
Dong, Guizhen, Dayton, NJ, UNITED STATES
Shao, Yuefei, Princeton, NJ, UNITED STATES
Zawacki, Lisa Guise, Yardley, PA, UNITED STATES
Yang, Cangmeng, Highland Park, NJ, UNITED STATES
Carroll, Carolyn Dilanni, Yardley, PA, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
PI US 7776862 B2 20100817
AI US 2006-353697 20060214 (11)
PRAI US 2005-653337P 20050216 (60)
DT Utility
FS GRANTED
LN.CNT 4037
INCL INCLM: 514/253.010
INCLS: 514/253.090; 514/253.100; 514/253.110; 514/253.130; 544/360.000
NCL NCLM: 514/253.010; 544/360.000
NCLS: 514/253.090; 514/253.100; 514/253.110; 514/253.130; 544/360.000
CPC CPCI C07D0401-12 [I]; C07D0211-58 [I]; C07D0401-14 [I]; C07D0405-14 [I]; C07D0413-14 [I]; C07D0417-14 [I]
CPCI-2 C07D0401-12 [I]; C07D0211-58 [I]; C07D0401-14 [I]; C07D0405-14 [I]; C07D0413-14 [I]; C07D0417-14 [I]
IPC IPCI C07D0401-00 [I]
IPCI-2 A61K0031-497 [I]; C07D0401-00 [I]
IPCR A61K0031-497 [I]; C07D0401-00 [I]
EXF 514/253.11; 514/253.13; 514/253.01; 514/253.09; 514/253.1; 544/360
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 232 OF 256 USPAT2 on STN
Full Text
AN 2007:18124 USPAT2
TI Pharmaceutical propylene glycol solvate compositions
IN Tawa, Mark, Brighton, MA, UNITED STATES
Almarsson, Orn, Shrewsbury, MA, UNITED STATES
Remenar, Julius, Framingham, MA, UNITED STATES
PA McNeil-PPC, Inc., Skillman, NJ, UNITED STATES (U.S. corporation)
PI US 7790905 B2 20100907
AI US 2003-747742 20031229 (10)
PRAI US 2003-486713P 20030711 (60)
US 2003-459501P 20030401 (60)
US 2003-456608P 20030321 (60)
US 2003-456027P 20030318 (60)
US 2003-441335P 20030121 (60)
US 2002-437516P 20021230 (60)
DT Utility
FS GRANTED
LN.CNT 17727
INCL INCLM: 548/375.100
INCLS: 514/406.000
NCL NCLM: 548/375.100; 514/738.000
CPC CPCI C07D0495-04 [I]

CPCI-2 C07D0495-04 [I]
IPC IPCI A61K0031-045 [I]
IPCI-2 C07D0231-12 [I]; A61K0031-415 [I]
IPCR C07D0231-12 [I]; A61K0031-415 [I]
EXF 424/246; 514/220; 514/557; 514/406; 548/375.1
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 233 OF 256 USPAT2 on STN
Full Text
AN 2007:11103 USPAT2
TI Methods for treating immune disorders associated with graft transplantation with soluble CTLA4 mutant molecules
IN Hagerty, David, Pennington, NJ, UNITED STATES
Rusnak, James, Newtown, PA, UNITED STATES
PA Bristol-Myers Squibb Company, Princeton, NJ, UNITED STATES (U.S. corporation)
PI US 7482327 B2 20090127
AI US 2006-399666 20060406 (11)
PRAI US 2005-668774P 20050406 (60)
DT Utility
FS GRANTED
LN.CNT 4097
INCL INCLM: 514/012.000
INCLS: 424/134.100
NCL NCLM: 514/001.100; 424/133.100
NCLS: 424/134.100; 424/185.100
CPC CPCI A61K0031-00 [I]; A61K0009-0019; A61K0031-436 [I]; A61K0031-5377 [I]; A61K0031-573 [I]; A61K0038-1774 [I]; A61K0039-395 [I]; C07K2319-30
CPCI-2 A61K0031-00 [I]; A61K0009-0019; A61K0031-436 [I]; A61K0031-5377 [I]; A61K0031-573 [I]; A61K0038-1774 [I]; A61K0039-395 [I]; C07K2319-30
IPC IPCI A61K0039-395 [I]; A61K0039-00 [I]
IPCI-2 A61K0038-16 [I]
IPCR A61K0038-16 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 234 OF 256 USPAT2 on STN
Full Text
AN 2006:327552 USPAT2
TI Modulation of NKG2D for treating or preventing solid organ allograft rejection
IN Kang, Sang-Mo, San Francisco, CA, UNITED STATES
Lanier, Lewis L., San Francisco, CA, UNITED STATES
PA The Regents of the University of California, Oakland, CA, UNITED STATES (U.S. corporation)
PI US 7998481 B2 20110816
AI US 2006-429354 20060505 (11)
RLI Continuation-in-part of Ser. No. WO 2005-US11487, filed on 5 Apr 2005, PENDING
PRAI US 2005-659678P 20050307 (60)
US 2004-576242P 20040601 (60)
US 2004-559919P 20040405 (60)
DT Utility
FS GRANTED
LN.CNT 3392
INCL INCLM: 424/139.100
INCLS: 424/577.000; 514/885.000
NCL NCLM: 424/139.100; 424/204.100
NCLS: 424/577.000; 514/885.000; 424/130.100
CPC CPCI C07K0016-2851 [I]; A61K2039-505; C07K2316-96; C07K2317-73; Y10S0514-885
CPCI-2 C07K0016-2851 [I]; A61K2039-505; C07K2316-96; C07K2317-73; Y10S0514-885
IPC IPCI A61K0039-395 [I]; A61K0039-12 [I]
IPCI-2 A61K0039-40 [I]; A61K0035-28 [I]; A01N0025-00 [I]
IPCR A61K0039-40 [I]; A01N0025-00 [I]; A61K0035-28 [I]

L2 ANSWER 235 OF 256 USPAT2 on STN
Full Text
AN 2006:322435 USPAT2
TI Amine-linked pyridyl and phenyl substituted piperazine-piperidines with

CXCR3 antagonist activity
IN Wong, Michael K. C., North Brunswick, NJ, UNITED STATES
Shu, Youheng, Blue Bell, PA, UNITED STATES
Yu, Wensheng, Edison, NJ, UNITED STATES
Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Kozlowski, Joseph A., Princeton, NJ, UNITED STATES
McGuinness, Brian F., Plainsboro, NJ, UNITED STATES
Shao, Yuefei, Princeton, NJ, UNITED STATES
Hobbs, Douglas W., Yardley, PA, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
Pharmacopeia Drug Discovery, Inc., Princeton, NJ, UNITED STATES (U.S. corporation)
PI US 7566718 B2 20090728
AI US 2006-354328 20060214 (11)
PRAI US 2005-653339P 20050216 (60)
DT Utility
FS GRANTED
LN.CNT 3775
INCL INCLM: 514/253.010
INCLS: 544/360.000; 544/364.000
NCL NCLM: 514/253.010
NCLS: 544/360.000; 544/364.000
CPC CPCI C07D0401-14 [I]; C07D0211-58 [I]; C07D0401-12 [I]; C07D0405-14 [I]; C07D0409-14 [I]; C07D0413-14 [I]; C07D0417-14 [I]
CPCI-2 C07D0401-14 [I]; C07D0211-58 [I]; C07D0401-12 [I]; C07D0405-14 [I]; C07D0409-14 [I]; C07D0413-14 [I]; C07D0417-14 [I]
IPC IPCI A61K0031-496 [I]; C07D0403-14 [I]
IPCI-2 A61K0031-497 [I]; C07D0401-00 [I]; C07D0403-00 [I]; C07D0405-00 [I]; C07D0409-00 [I]
IPCR A61K0031-497 [I]; C07D0401-00 [I]; C07D0403-00 [I]; C07D0405-00 [I]; C07D0409-00 [I]
EXF 514/253.01; 544/364
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 236 OF 256 USPAT2 on STN
Full Text
AN 2006:322434 USPAT2
TI Heterocyclic substituted piperazines with CXCR3 antagonist activity
IN Kim, Seong Heon, Livingston, NJ, UNITED STATES
Anilkumar, Gopinadhan N., Edison, NJ, UNITED STATES
Wong, Michael K. C., North Brunswick, NJ, UNITED STATES
Zeng, Qingbei, Edison, NJ, UNITED STATES
Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Kozlowski, Joseph A., Princeton, NJ, UNITED STATES
Shao, Yuefei, Princeton, NJ, UNITED STATES
McGuinness, Brian F., Plainsboro, NJ, UNITED STATES
Hobbs, Douglas W., Yardley, PA, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
Pharmacopeia Drug Discovery, Inc., Princeton, NJ, UNITED STATES (U.S. corporation)
PI US 7868006 B2 20110111
AI US 2006-353641 20060214 (11)
PRAI US 2005-653309P 20050216 (60)
DT Utility
FS GRANTED
LN.CNT 3895
INCL INCLM: 514/252.120
INCLS: 544/357.000
NCL NCLM: 514/252.120
NCLS: 544/357.000
CPC CPCI C07D0401-12 [I]; C07D0401-14 [I]; C07D0413-14 [I]; C07D0417-14 [I]
CPCI-2 C07D0401-12 [I]; C07D0401-14 [I]; C07D0413-14 [I]; C07D0417-14 [I]
IPC IPCI A61K0031-497 [I]; C07D0413-14 [I]; C07D0403-14 [I]
IPCI-2 A61K0031-497 [I]; C07D0241-02 [I]
IPCR A61K0031-497 [I]; C07D0241-02 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 237 OF 256 USPAT2 on STN
Full Text
AN 2006:322412 USPAT2

TI Piperazine-piperidines with CXCR3 antagonist activity
IN Yu, Wensheng, Edison, NJ, UNITED STATES
Kim, Seong Heon, Livingston, NJ, UNITED STATES
Anilkumar, Gopinadhan N., Edison, NJ, UNITED STATES
Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Shankar, Bandarpalle B., Branchburg, NJ, UNITED STATES
McGuinness, Brian F., Plainsboro, NJ, UNITED STATES
Hobbs, Douglas W., Yardley, PA, UNITED STATES
Shao, Yuefei, Princeton, NJ, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
PI US 7763616 B2 20100727
AI US 2006-353806 20060214 (11)
PRAI US 2005-653378P 20050216 (60)
DT Utility
FS GRANTED
LN.CNT 2577
INCL INCLM: 514/249.000
INCLS: 540/460.000; 540/502.000; 540/557.000; 544/350.000; 544/258.000; 544/357.000; 544/229.000; 544/393.000; 544/360.000; 544/364.000; 544/354.000; 514/253.010; 514/183.000; 514/221.000; 514/220.000; 514/253.090
NCL NCLM: 514/249.000; 514/221.000
NCLS: 514/183.000; 514/220.000; 514/221.000; 514/253.010; 514/253.090; 540/460.000; 540/502.000; 540/557.000; 544/229.000; 544/258.000; 544/350.000; 544/354.000; 544/357.000; 544/360.000; 544/364.000; 544/393.000; 514/251.000; 544/256.000
CPC CPCI C07D0487-04 [I]; C07D0401-12 [I]
CPCI-2 C07D0487-04 [I]; C07D0401-12 [I]
IPC IPCI A61K0031-551 [I]; A61K0031-498 [I]; A61K0031-496 [I]; A61K0031-525 [I]; C07D0487-02 [I]; C07D0475-02 [I]
IPCI-2 A61K0031-5025 [I]; A61K0031-4985 [I]; C07D0245-02 [I]; C07D0255-02 [I]; C07D0257-08 [I]; C07D0259-00 [I]; C07D0239-04 [I]; C07D0239-70 [I]; C07D0471-04 [I]; C07D0487-04 [I]; A61K0031-497 [N]; C07D0241-36 [N]; C07D0401-14 [N]; C07D0403-14 [N]; C07D0405-14 [N]; C07D0409-14 [N]; A61P0029-00 [N]; A61P0017-06 [N]; A61P0019-02 [N]; A61P0003-10 [N]; A61P0031-08 [N]
IPCR A61K0031-5025 [I]; A61K0031-497 [N]; A61K0031-4985 [I]; A61P0003-10 [N]; A61P0017-06 [N]; A61P0019-02 [N]; A61P0029-00 [N]; A61P0031-08 [N]; C07D0239-04 [I]; C07D0239-70 [I]; C07D0241-36 [N]; C07D0245-02 [I]; C07D0255-02 [I]; C07D0257-08 [I]; C07D0259-00 [I]; C07D0401-14 [N]; C07D0403-14 [N]; C07D0405-14 [N]; C07D0409-14 [N]; C07D0471-04 [I]; C07D0487-04 [I]
EXF 514/249; 514/183; 514/221; 514/220; 540/460; 540/501; 540/502; 540/557; 544/350; 544/249; 544/258
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 238 OF 256 USPAT2 on STN
Full Text
AN 2006:322403 USPAT2
TI Heteroaryl substituted pyrazinyl-piperazine-piperidines with CXCR3 antagonist activity
IN Zeng, Qingbei, Edison, NJ, UNITED STATES
Yang, De-Yi, Morris Plains, NJ, UNITED STATES
Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Wong, Michael K. C., North Brunswick, NJ, UNITED STATES
Anilkumar, Gopinadhan N., Edison, NJ, UNITED STATES
Kim, Seong Heon, Livingston, NJ, UNITED STATES
Yu, Wensheng, Edison, NJ, UNITED STATES
Kozlowski, Joseph A., Princeton, NJ, UNITED STATES
Shih, Neng-Yang, Warren, NJ, UNITED STATES
McGuinness, Brian F., Plainsboro, NJ, UNITED STATES
Zawacki, Lisa Guise, Yardley, PA, UNITED STATES
Hobbs, Doublas W., Yardley, PA, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
Pharmacopeia Drug Discovery, Inc., Princeton, NJ, UNITED STATES (U.S. corporation)
PI US 7879838 B2 20110201
AI US 2006-353474 20060214 (11)
PRAI US 2005-653477P 20050216 (60)
DT Utility

FS GRANTED
LN.CNT 3361
INCL INCLM: 514/210.200
INCLS: 514/235.500; 514/252.110; 544/120.000; 544/295.000; 544/357.000
NCL NCLM: 514/210.200
NCLS: 514/235.500; 514/252.110; 544/120.000; 544/295.000; 544/357.000
CPC CPCI C07D0401-12 [I]; C07D0401-14 [I]; C07D0405-14 [I]; C07D0409-14 [I]; C07D0413-14 [I]; C07D0417-14 [I]; C07D0487-04 [I]
CPCI-2 C07D0401-12 [I]; C07D0401-14 [I]; C07D0405-14 [I]; C07D0409-14 [I]; C07D0413-14 [I]; C07D0417-14 [I]; C07D0487-04 [I]
IPC IPCI A61K0031-5377 [I]; A61K0031-506 [I]; A61K0031-497 [I]; C07D0413-14 [I]; C07D0403-14 [I]
IPCI-2 A01N0043-00 [I]; A61K0031-00 [I]; A61K0031-535 [I]; A61K0031-497 [I]; C07D0413-00 [I]; C07D0403-00 [I]; C07D0241-02 [I]
IPCR A01N0043-00 [I]; A61K0031-00 [I]; A61K0031-497 [I]; A61K0031-535 [I]; C07D0241-02 [I]; C07D0403-00 [I]; C07D0413-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 239 OF 256 USPAT2 on STN
Full Text
AN 2006:308790 USPAT2
TI Aminopropanol derivatives
IN Albert, Rainer, Basel, SWITZERLAND
Francotte, Eric, Nuglar, SWITZERLAND
Zecri, Frederic, Bartenheim, FRANCE
Zollinger, Markus, Mohlin, SWITZERLAND
PA Novartis AG, Basel, SWITZERLAND (non-U.S. corporation)
PI US 7528120 B2 20090505
WO 2005021503 20050310
AI US 2006-569696 20040827 (10)
WO 2004-EP9589 20040827
20060227 PCT 371 date
PRAI GB 2003-20196 20030828
GB 2003-24206 20031015
DT Utility
FS GRANTED
LN.CNT 1100
INCL INCLM: 514/080.000
INCLS: 548/414.000
NCL NCLM: 514/080.000
NCLS: 548/414.000
CPC CPCI C07F0009-5728 [I]; C07D0209-46 [I]; C07D0263-14 [I]; C07F0009-094 [I]; C07F0009-653 [I]
CPCI-2 C07F0009-5728 [I]; C07D0209-46 [I]; C07D0263-14 [I]; C07F0009-094 [I]; C07F0009-653 [I]
IPC IPCI A61K0031-675 [I]; C07F0009-547 [I]
IPCI-2 A61K0031-675 [I]; C07F0009-572 [I]
IPCR A61K0031-675 [I]; A61P0037-02 [I]; C07D0209-46 [I]; C07D0263-14 [I]; C07F0009-09 [I]; C07F0009-572 [I]; C07F0009-653 [I]
EXF 548/414; 548/470; 548/472; 514/80
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 240 OF 256 USPAT2 on STN
Full Text
AN 2006:254936 USPAT2
TI Heterocyclic substituted pyridine or phenyl compounds with CXCR3 antagonist activity
IN Anilkumar, Gopinadhan N., Edison, NJ, UNITED STATES
Zeng, Qingbei, Edison, NJ, UNITED STATES
Rosenblum, Stuart B., West Orange, NJ, UNITED STATES
Kozlowski, Joseph A., Princeton, NJ, UNITED STATES
McGuinness, Brian F., Plainsboro, NJ, UNITED STATES
Hobbs, Douglas W., Yardley, PA, UNITED STATES
PA Schering Corporation, Kenilworth, NJ, UNITED STATES (U.S. corporation)
Pharmacopeia Inc., Princeton, NJ, UNITED STATES (U.S. corporation)
PI US 7417045 B2 20080826
AI US 2006-353609 20060214 (11)
PRAI US 2005-653332P 20050216 (60)
DT Utility
FS GRANTED
LN.CNT 3506
INCL INCLM: 514/252.120

INCLS: 544/358.000; 544/359.000
NCL NCLM: 514/253.010
NCLS: 544/364.000
CPC CPCI C07D0417-14 [I]; C07D0401-04 [I]; C07D0401-14 [I]; C07D0413-14 [I]
IPC IPCI C07D0403-14 [I]; A61K0031-496 [I]
IPCR C07D0403-14 [I]; A61K0031-496 [I]
EXF 544/358; 544/359; 514/252.12
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 241 OF 256 USPAT2 on STN
Full Text
AN 2006:215518 USPAT2
TI Pharmaceutical composition consisting of rapamycine and gastrin 17(LEU15) and a method for treating diabetes
IN Brand, Stephen J., Lincoln, MA, UNITED STATES
Cruz, Antonio, Toronto, CANADA
PA Waratah Pharmaceuticals Inc., Toronto, Ontario, CANADA (non-U.S. corporation)
PI US 7560425 B2 20090714
WO 2003103701 20031218
AI US 2006-517135 20030609 (10)
WO 2003-US18377 20030609
20060217 PCT 371 date
DT Utility
FS GRANTED
LN.CNT 1920
INCL INCLM: 514/002.000
INCLS: 530/300.000; 530/309.000; 424/198.100; 524/026.000
NCL NCLM: 514/001.100; 514/009.600
NCLS: 424/198.100; 514/006.900; 514/012.300; 514/012.600; 524/026.000; 530/300.000; 530/309.000; 514/109.000; 514/171.000; 514/232.500; 514/251.000; 514/263.310; 514/291.000; 514/651.000
CPC CPCI A61K0038-2207 [I]; A61K0031-436 [I]; A61K0031-522 [I]; A61K0031-525 [I]; A61K0031-535 [I]; A61K0031-5377 [I]; A61K0031-573 [I]; A61K0031-663 [I]; A61K0038-1808 [I]; A61K0038-21 [I]; A61K0038-28 [I]; A61K0045-06 [I]
CPCI-2 A61K0038-2207 [I]; A61K0031-436 [I]; A61K0031-522 [I]; A61K0031-525 [I]; A61K0031-535 [I]; A61K0031-5377 [I]; A61K0031-573 [I]; A61K0031-663 [I]; A61K0038-1808 [I]; A61K0038-21 [I]; A61K0038-28 [I]; A61K0045-06 [I]
IPC IPCI A61K0038-18 [I]; A61K0038-13 [I]; A61K0031-522 [I]; A61K0031-5377 [I]; A61K0031-663 [I]; A61K0031-573 [I]; A61K0031-535 [I]; A61K0031-525 [I]
IPCI-2 A61K0038-00 [I]; A01N0045-00 [I]
IPCR A61K0038-00 [I]; A61K0045-06 [I]; A01N0045-00 [I]; A61K0031-133 [I]; A61K0031-135 [I]; A61K0031-136 [I]; A61K0031-14 [I]; A61K0031-343 [I]; A61K0031-436 [I]; A61K0031-519 [I]; A61K0031-52 [I]; A61K0031-573 [I]; A61K0031-675 [I]; A61K0038-18 [I]; A61K0038-21 [I]; A61K0038-22 [I]; A61K0038-28 [I]; A61K0039-395 [I]; A61K0048-00 [N]; A61P0003-10 [I]; A61P0037-06 [I]; A61P0043-00 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 242 OF 256 USPAT2 on STN
Full Text
AN 2005:268813 USPAT2
TI Medicinal compositions for preventing or treating viral myocarditis
IN Matsumori, Akira, Minoh, JAPAN
PA Mitsubishi Tanabe Pharma Corporation, Osaka, JAPAN (non-U.S. corporation)
PI US 7902261 B2 20110308
AI US 2005-139532 20050531 (11)
RLI Continuation of Ser. No. US 1900-19263, ABANDONED A 371 of International Ser. No. WO 2000-JP4286, filed on 28 Jun 2000
PRAI JP 1999-185297 19990630
DT Utility
FS GRANTED
LN.CNT 600
INCL INCLM: 514/727.000
NCL NCLM: 514/727.000; 514/651.000
CPC CPCI A61K0031-137 [I]

CPCI-2 A61K0031-137 [I]
IPC IPCI A61K0031-138 [ICM,7]
IPCI-2 A01N0033-18 [I]; A01N0033-24 [I]
IPCR A01N0033-18 [I]; A61K0031-133 [I]; A01N0033-24 [I]; A61K0031-137 [I]; A61P0009-04 [I]; A61P0031-12 [I]; A61P0031-14 [I]; A61P0031-16 [I]; A61P0031-22 [I]
EXF 514/726; 514/727
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 243 OF 256 USPAT2 on STN
Full Text
AN 2005:38064 USPAT2
TI Method for alleviating pain using sphingosine-1-phosphate and related compounds, and assays for identifying such compounds
IN Michaelis, Martin, Frankfurt, GERMANY, FEDERAL REPUBLIC OF
Geisslinger, Gerd, Bad Soden, GERMANY, FEDERAL REPUBLIC OF
Scholich, Klaus, Dreieich, GERMANY, FEDERAL REPUBLIC OF
PA Sanofi-Aventis Pharma GmbH, Frankfurt am Main, GERMANY, FEDERAL REPUBLIC OF (non-U.S. corporation)
PI US 7691563 B2 20100406
AI US 2004-850586 20040520 (10)
PRAI EP 2003-12389 20030530
US 2003-520780P 20031117 (60)
DT Utility
FS GRANTED
LN.CNT 9286
INCL INCLM: 435/004.000
NCL NCLM: 435/004.000; 514/054.000
NCLS: 514/078.000
CPC CPCI A61K0031-661 [I]; A61K0031-685 [I]; C07F0009-113 [I]; G01N0033-5008 [I]; G01N0033-5023 [I]; G01N0033-5058 [I]; G01N0033-6896 [I]; G01N0033-92 [I]; G01N2333-988; G01N2500-02; G01N2500-04; G01N2500-10
CPCI-2 A61K0031-661 [I]; A61K0031-685 [I]; C07F0009-113 [I]; G01N0033-5008 [I]; G01N0033-5023 [I]; G01N0033-5058 [I]; G01N0033-6896 [I]; G01N0033-92 [I]; G01N2333-988; G01N2500-02; G01N2500-04; G01N2500-10
IPC IPCI A61K0031-685 [ICM,7]
IPCI-2 C12Q0001-00 [I]
IPCR C12Q0001-00 [I]; A61K0031-661 [I]; A61K0031-685 [I]; A61P0029-00 [I]; C07F0009-113 [I]; G01N0033-50 [I]; G01N0033-68 [I]; G01N0033-92 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 244 OF 256 USPAT2 on STN
Full Text
AN 2004:320576 USPAT2
TI Methods of suppressing, treating, or preventing graft rejection with an antibody or a portion thereof that binds to AILIM
IN Suzuki, legal representative, Atsuko, Tokyo, JAPAN
Isobe, Mitsuaki, Tokyo, JAPAN
Suzuki, Seiichi, United States deceased
Suzuki, Seiichi, Tokyo, JAPAN
PA Japan Tobacco, Inc., Tokyo, JAPAN (non-U.S. corporation)
PI US 7438905 B2 20081021
AI US 2004-793171 20040304 (10)
RLI Continuation-in-part of Ser. No. US 2002-451972, ABANDONED A 371 of International Ser. No. WO 2002-JP930, filed on 5 Feb 2002
PRAI JP 2001-56209 20010301
JP 2001-56216 20010301
JP 2002-8028 20020116
DT Utility
FS GRANTED
LN.CNT 2032
INCL INCLM: 424/130.100
INCLS: 424/133.100; 424/135.100; 424/141.100; 424/142.100; 424/154.100; 530/387.100; 530/388.100; 530/388.150; 530/388.220
NCL NCLM: 424/130.100; 424/131.100
NCLS: 424/133.100; 424/135.100; 424/141.100; 424/142.100; 424/154.100; 530/387.100; 530/388.100; 530/388.150; 530/388.220; 514/020.500; 514/291.000
CPC CPCI C07K0016-2818 [I]; A61K0038-00; A61K2039-505; C07K0016-28 [I];

C07K2319-30
CPCI-2 C07K0016-2818 [I]; A61K0038-00; A61K2039-505; C07K0016-28 [I]; C07K2319-30
IPC IPCI A61K0039-395 [ICM,7]; A61K0038-13 [ICS,7]; A61K0031-4745 [ICS,7]
IPCI-2 A61K0039-395 [I]; C07K0016-00 [I]; C07K0016-28 [I]
IPCR A61K0038-02 [I]; A61K0039-395 [I]; A61K0031-7105 [I]; A61K0031-711 [I]; A61K0038-00 [I]; A61K0045-00 [I]; A61P0037-06 [I]; C07K0016-00 [I]; C07K0016-28 [I]
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 245 OF 256 USPAT2 on STN
Full Text
AN 2004:315311 USPAT2
TI 2-amino-1,3-propanediol compounds for the treatment of acute pain
IN Michaelis, Martin, Frankfurt, GERMANY, FEDERAL REPUBLIC OF
Geisslinger, Gerd, Bad Soden, GERMANY, FEDERAL REPUBLIC OF
Scholich, Klaus, Dreieich, GERMANY, FEDERAL REPUBLIC OF
PA Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, GERMANY, FEDERAL REPUBLIC OF (non-U.S. corporation)
PI US 7524887 B2 20090428
AI US 2004-853761 20040525 (10)
PRAI EP 2003-12864 20030606
US 2003-510994P 20031014 (60)
DT Utility
FS GRANTED
LN.CNT 939
INCL INCLM: 514/653.000
INCLS: 514/667.000; 564/360.000; 564/503.000; 564/507.000
NCL NCLM: 514/653.000; 514/649.000
NCLS: 514/667.000; 564/360.000; 564/503.000; 564/507.000
CPC CPCI A61K0031-137 [I]
CPCI-2 A61K0031-137 [I]
IPC IPCI A61K0031-137 [ICM,7]
IPCI-2 A61K0031-137 [I]; A61K0031-131 [I]; A61K0031-132 [I]; C07C0215-10 [I]; C07C0215-20 [I]; C07C0217-28 [I]; C07C0217-44 [I]; C07C0219-06 [I]; C07C0219-04 [I]; C07C0219-18 [I]
IPCR A61K0031-137 [I]; A61K0031-131 [I]; A61K0031-132 [I]; C07C0215-10 [I]; C07C0215-20 [I]; C07C0217-28 [I]; C07C0217-44 [I]; C07C0219-04 [I]; C07C0219-06 [I]; C07C0219-18 [I]
EXF 514/255; 514/357; 514/427
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 246 OF 256 USPAT2 on STN
Full Text
AN 2004:165963 USPAT2
TI Method for treating diseases associated with abnormal kinase activity
IN Lyons, John, Moraga, CA, UNITED STATES
Rubinfeld, Joseph, Danville, CA, UNITED STATES
PA SuperGen.Inc., Dublin, CA, UNITED STATES (U.S. corporation)
PI US 6998391 B2 20060214
AI US 2002-206854 20020726 (10)
RLI Continuation-in-part of Ser. No. US 2002-71849, filed on 7 Feb 2002, ABANDONED
DT Utility
FS GRANTED
LN.CNT 1784
INCL INCLM: 514/049.000
INCLS: 514/085.000; 514/269.000; 514/234.500; 514/300.000; 536/023.500; 536/023.100; 435/069.100; 435/325.000; 530/350.000; 424/045.000; 424/450.000
NCL NCLM: 514/049.000; 514/050.000
NCLS: 424/045.000; 424/450.000; 435/069.100; 435/325.000; 514/085.000; 514/234.500; 514/269.000; 514/300.000; 530/350.000; 536/023.100; 536/023.500
CPC CPCI A61K0031-7068 [I]; A61K0031-00 [I]; A61K0031-706 [I]; A61K0045-06 [I]
CPCI-2 A61K0031-7068 [I]; A61K0031-00 [I]; A61K0031-706 [I]; A61K0045-06 [I]
IPC IPCI A61K0031-7072 [ICM,7]
IPCI-2 A61K0031-7072 [I]
IPCR A61K0031-7072 [I]; A61K0031-00 [I]; A61K0031-706 [I]; A61K0031-7068 [I]

EXF 514/49; 514/65; 514/269; 514/234.5; 514/300; 536/23.5; 536/23.1; 435/69.1; 435/325; 530/350; 424/45; 424/450
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 247 OF 256 USPAT2 on STN
Full Text
AN 2003:325269 USPAT2
TI Production of 2-amino-2-[2- (4-alkylphenyl) ethyl] propane-1, 3-diols
IN Abel, Stephan, Weil, GERMANY, FEDERAL REPUBLIC OF
Fujita, Tetsuro, Kyoto, JAPAN
Hirose, Ryoji, Hyogo, JAPAN
Jordine, Guido, Freiburg, GERMANY, FEDERAL REPUBLIC OF
Mishina, Tadashi, Osaka, JAPAN
PA Novartis AG, Basel, SWEDEN (non-U.S. corporation)
PI US 7026522 B2 20060411
AI US 2003-464294 20030618 (10)
RLI Division of Ser. No. US 2001-850851, filed on 8 May 2001, Pat. No. US 6605744 Continuation of Ser. No. WO 1999-EP8577, filed on 9 Nov 1999, PENDING
PRAI GB 1998-24705 19981111
GB 1998-24706 19981111
DT Utility
FS GRANTED
LN.CNT 639
INCL INCLM: 570/195.000
INCLS: 570/194.000; 570/201.000
NCL NCLM: 570/195.000; 564/355.000
NCLS: 570/194.000; 570/201.000
CPC CPCI C07C0233-47 [I]; C07C0017-16 [I]; C07C0017-208 [I]; C07C0017-35 [I]; C07C0022-04 [I]; C07C0029-00 [I]; C07C0045-46 [I]; C07C0045-63 [I]; C07C0213-00 [I]; C07C0215-28 [I]
CPCI-2 C07C0233-47 [I]; C07C0017-16 [I]; C07C0017-208 [I]; C07C0017-35 [I]; C07C0022-04 [I]; C07C0029-00 [I]; C07C0045-46 [I]; C07C0045-63 [I]; C07C0213-00 [I]; C07C0215-28 [I]
IPC IPCI C07C0215-28 [ICM,7]
IPCI-2 C07C0022-00 [I]
IPCR C07C0022-00 [I]; C07C0017-16 [I]; C07C0017-20 [I]; C07C0017-35 [I]; C07C0022-04 [I]; C07C0029-00 [I]; C07C0045-46 [I]; C07C0045-63 [I]; C07C0213-00 [I]; C07C0213-02 [I]; C07C0215-28 [N]; C07C0215-34 [I]; C07C0231-12 [I]; C07C0233-05 [I]; C07C0233-47 [I]
EXF 570/195; 570/194; 570/201
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 248 OF 256 USPAT2 on STN
Full Text
AN 2003:201813 USPAT2
TI Delivery of therapeutic capable agents
IN Sirhan, Motasim, Sunnyvale, CA, UNITED STATES
Yan, John, Los Gatos, CA, UNITED STATES
PA Avantec Vascular Corporation, Sunnyvale, CA, UNITED STATES (U.S. corporation)
PI US 7083642 B2 20060801
AI US 2002-206853 20020725 (10)
RLI Continuation-in-part of Ser. No. US 2001-2595, filed on 1 Nov 2001, PENDING Continuation-in-part of Ser. No. US 2001-783253, filed on 13 Feb 2001, Pat. No. US 6939375 Continuation-in-part of Ser. No. US 2001-782927, filed on 13 Feb 2001, Pat. No. US 6471980 Continuation-in-part of Ser. No. US 2001-783254, filed on 13 Feb 2001, PENDING Continuation-in-part of Ser. No. US 2001-782804, filed on 13 Feb 2001, PENDING Continuation-in-part of Ser. No. US 2001-206853, PENDING Continuation-in-part of Ser. No. US 2001-17500, filed on 14 Dec 2001, PENDING
PRAI US 2002-370703P 20020406 (60)
US 2002-355317P 20020207 (60)
US 2002-347473P 20020110 (60)
US 2001-308381P 20010726 (60)
US 2000-258024P 20001222 (60)
DT Utility
FS GRANTED
LN.CNT 1239
INCL INCLM: 623/001.420

INCLS: 623/001.150
NCL NCLM: 623/001.420; 623/001.150
NCLS: 623/001.150
CPC CPCI A61L0027-54 [I]; A61F0002-91 [I]; A61F0002-915 [I]; A61F0002-95; A61F2002-91533; A61F2002-91558; A61F2250-0067; A61F2250-0071; A61L0031-16 [I]; A61L2300-416; A61L2300-602
CPCI-2 A61L0027-54 [I]; A61F0002-91 [I]; A61F0002-915 [I]; A61F0002-95; A61F2002-91533; A61F2002-91558; A61F2250-0067; A61F2250-0071; A61L0031-16 [I]; A61L2300-416; A61L2300-602
IPC IPCI A61F0002-06 [ICM,7]
IPCI-2 A61F0002-06 [I]
IPCR A61F0002-06 [I]; A61F0002-00 [N]; A61F0002-84 [N]; A61F0002-90 [I]; A61L0027-54 [I]; A61L0031-16 [I]
EXF 623/1.39-1.48; 623/23.29; 623/23.3
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 249 OF 256 USPAT2 on STN
Full Text
AN 2003:121162 USPAT2
TI Apparatus and methods for variably controlled substance delivery from implanted prostheses
IN Sirhan, Motasim, Sunnyvale, CA, UNITED STATES
Yan, John, Los Gatos, CA, UNITED STATES
PA Avantec Vascular Corporation, Sunnyvale, CA, UNITED STATES (U.S. corporation)
PI US 7077859 B2 20060718
AI US 2001-17500 20011214 (10)
RLI Continuation-in-part of Ser. No. US 2001-2595, filed on 1 Nov 2001, PENDING Continuation-in-part of Ser. No. US 2001-783253, filed on 13 Feb 2001, PENDING Continuation-in-part of Ser. No. US 2001-782927, filed on 13 Feb 2001, Pat. No. US 6471980 Continuation-in-part of Ser. No. US 2001-783254, filed on 13 Feb 2001, PENDING Continuation-in-part of Ser. No. US 2001-782804, filed on 13 Feb 2001, PENDING
PRAI US 2001-308381P 20010726 (60)
US 2000-258024P 20001222 (60)
DT Utility
FS GRANTED
LN.CNT 2448
INCL INCLM: 623/001.150
INCLS: 623/001.420
NCL NCLM: 623/001.150; 604/891.100
NCLS: 623/001.420
CPC CPCI A61L0027-54 [I]; A61F0002-91 [I]; A61F0002-915 [I]; A61F0002-95; A61F2002-91533; A61F2002-91558; A61F2002-91583; A61F2250-0067; A61F2250-0068; A61L0031-16 [I]; A61L2300-416; A61L2300-602
CPCI-2 A61L0027-54 [I]; A61F0002-91 [I]; A61F0002-915 [I]; A61F0002-95; A61F2002-91533; A61F2002-91558; A61F2002-91583; A61F2250-0067; A61F2250-0068; A61L0031-16 [I]; A61L2300-416; A61L2300-602
IPC IPCI A61F0002-06 [ICM,7]
IPCI-2 A61F0002-06 [I]
IPCR A61F0002-06 [I]; A61K0009-00 [I]; A61F0002-00 [N]; A61F0002-82 [I]; A61F0002-84 [N]; A61F0002-90 [I]; A61L0027-54 [I]; A61L0031-00 [I]; A61L0031-16 [I]
EXF 623/1.13; 623/1.39; 623/1.4-1.48
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 250 OF 256 USPAT2 on STN
Full Text
AN 2003:23354 USPAT2
TI Intravascular delivery of mycophenolic acid
IN Sirhan, Motasim, Sunnyvale, CA, United States
Yan, John, Los Gatos, CA, United States
PA Avantec Vascular Corporation, Sunnyvale, CA, United States (U.S. corporation)
PI US 6858221 B2 20050222
AI US 2002-242334 20020911 (10)
RLI Division of Ser. No. US 2001-782927, filed on 13 Feb 2001, now patented, Pat. No. US 6471980
PRAI US 2000-258024P 20001222 (60)
DT Utility
FS GRANTED
LN.CNT 1119

INCL INCLM: 424/423.000
INCLS: 424/424.000; 424/425.000; 424/426.000; 623/001.100; 623/001.420
NCL NCLM: 424/423.000; 424/426.000
NCLS: 424/424.000; 424/425.000; 424/426.000; 623/001.100; 623/001.420; 514/171.000; 514/251.000; 514/291.000; 514/470.000
CPC CPCI A61F0002-90 [I]; A61F0002-91 [I]; A61F0002-915 [I]; A61F0002-95; A61F2002-91533; A61F2250-0067; A61L0027-54 [I]; A61L0031-16 [I]; A61L2300-416; A61L2300-602
CPCI-2 A61F0002-90 [I]; A61F0002-91 [I]; A61F0002-915 [I]; A61F0002-95; A61F2002-91533; A61F2250-0067; A61L0027-54 [I]; A61L0031-16 [I]; A61L2300-416; A61L2300-602
IPC [7]
IPCI A61K0031-573 [ICM,7]; A61K0031-525 [ICS,7]; A61K0031-4745 [ICS,7]; A61F0002-00 [ICS,7]; A61K0031-365 [ICS,7]
IPCI-2 A61F0002-02 [ICM,7]; A61F0002-06 [ICS,7]
IPCR A61F0002-00 [N]; A61F0002-06 [I]; A61F0002-84 [N]; A61F0002-90 [I]; A61L0027-54 [I]; A61L0031-16 [I]
EXF 424/423; 424/424; 424/425; 424/426; 623/1.1; 623/1.42
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 251 OF 256 USPAT2 on STN
Full Text
AN 2002:213450 USPAT2
TI Intravascular delivery of mycophenolic acid
IN Sirhan, Motasim, Sunnyvale, CA, United States
Yan, John, Los Gatos, CA, United States
PA Avantec Vascular Corporation, Sunnyvale, CA, United States (U.S. corporation)
PI US 6471980 B2 20021029
AI US 2001-782927 20010213 (9)
PRAI US 2000-258024P 20001222 (60)
DT Utility
FS GRANTED
LN.CNT 1071
INCL INCLM: 424/423.000
INCLS: 424/424.000; 424/425.000; 424/426.000
NCL NCLM: 424/423.000
NCLS: 424/424.000; 424/425.000; 424/426.000; 514/470.000
CPC CPCI A61F0002-90 [I]; A61F0002-91 [I]; A61F0002-915 [I]; A61F0002-95; A61F2002-91533; A61F2250-0067; A61L0027-54 [I]; A61L0031-16 [I]; A61L2300-416; A61L2300-602
CPCI-2 A61F0002-90 [I]; A61F0002-91 [I]; A61F0002-915 [I]; A61F0002-95; A61F2002-91533; A61F2250-0067; A61L0027-54 [I]; A61L0031-16 [I]; A61L2300-416; A61L2300-602
IPC [7]
IPCI A61F0002-00 [ICM,7]; A61K0031-365 [ICS,7]
IPCI-2 A61F0002-02 [ICM,7]
IPCR A61F0002-00 [N]; A61F0002-06 [I]; A61F0002-84 [N]; A61F0002-90 [I]; A61L0027-54 [I]; A61L0031-16 [I]
EXF 424/423; 424/424; 424/425; 424/426
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 252 OF 256 USPAT2 on STN
Full Text
AN 2002:191216 USPAT2
TI Compositions and methods of using compositions with accelerated lymphocyte homing immunosuppressive properties
IN Chiba, Kenji, Fukuoka, JAPAN
Adachi, Kunitomo, Fukuoka, JAPAN
PA Mitsubishi Pharma Corporation, Osaka, JAPAN (non-U.S. corporation)
PI US 6667025 B2 20031223
AI US 1999-334213 19990615 (9)
RLI Division of Ser. No. US 1997-933738, filed on 23 Sep 1997, now patented, Pat. No. US 6004565
PRAI JP 1997-237273 19970902
DT Utility
FS GRANTED
LN.CNT 1499
INCL INCLM: 424/009.100
INCLS: 424/009.200; 424/093.700; 424/278.100; 435/002.000; 435/029.000; 514/885.000
NCL NCLM: 424/009.100; 424/278.100

NCLS: 424/009.200; 424/093.700; 424/278.100; 435/002.000; 435/029.000; 514/885.000; 514/472.000; 514/487.000; 514/546.000; 514/653.000
CPC CPCI A61K0031-135 [I]; Y10S0514-885
CPCI-2 A61K0031-135 [I]; Y10S0514-885
IPC [7]
IPCI A61K0045-00 [ICM,7]; A61K0047-00 [ICS,7]; A61K0031-34 [ICS,7]; A01N0043-08 [ICS,7]; A61K0031-27 [ICS,7]; A01N0047-10 [ICS,7]; A61K0031-22 [ICS,7]; A01N0037-02 [ICS,7]; A61K0031-135 [ICS,7]; A01N0033-02 [ICS,7]
IPCI-2 A61K0049-00 [ICM,7]; A61K0045-00 [ICS,7]; A01N0001-02 [ICS,7]
IPCR G01N0033-49 [I]; A61K0031-13 [I]; A61K0031-135 [I]; A61K0045-00 [I]; A61P0037-06 [I]
EXF 424/278.1; 424/9.1; 424/9.2; 424/93.7; 514/472; 514/653; 514/487; 514/546; 514/885; 560/29; 560/163; 564/223; 564/355; 435/2; 435/29
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 253 OF 256 USPAT2 on STN
Full Text
AN 2002:172348 USPAT2
TI Phosphate derivatives as immunoregulatory agents
IN Mandala, Suzanne, Scotch Plains, NJ, United States
Bergstrom, James, Neshanic Station, NJ, United States
Hajdu, Richard, Old Bridge, NJ, United States
Rosen, Hugh, Springfield, NJ, United States
Parsons, William, Belle Mead, NJ, United States
Card, Deborah J., Somerset, NJ, United States
Maccoss, Malcolm, Freehold, NJ, United States
Kathleen, Rupprecht, Cranford, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 6437165 B2 20020820
AI US 2001-942411 20010830 (9)
PRAI US 2000-229438P 20000831 (60)
DT Utility
FS GRANTED
LN.CNT 1329
INCL INCLM: 558/169.000
INCLS: 558/070.000; 558/166.000; 514/114.000
NCL NCLM: 558/169.000; 514/114.000
NCLS: 558/070.000; 558/166.000; 514/118.000; 558/116.000
CPC CPCI C07F0009-094 [I]; C07F0009-3882 [I]
CPCI-2 C07F0009-094 [I]; C07F0009-3882 [I]
IPC [7]
IPCI A61K0031-66 [ICM,7]; C07F0009-24 [ICS,7]
IPCI-2 A61K0031-662 [ICM,7]
IPCR A61K0031-661 [I]; A61K0031-662 [I]; A61P0001-02 [I]; A61P0001-04 [I]; A61P0001-16 [I]; A61P0001-18 [I]; A61P0003-10 [I]; A61P0005-14 [I]; A61P0007-00 [I]; A61P0007-06 [I]; A61P0009-04 [I]; A61P0009-10 [I]; A61P0011-00 [I]; A61P0011-02 [I]; A61P0011-06 [I]; A61P0013-12 [I]; A61P0017-00 [I]; A61P0017-04 [I]; A61P0017-06 [I]; A61P0017-10 [I]; A61P0017-14 [I]; A61P0019-02 [I]; A61P0021-00 [I]; A61P0025-00 [I]; A61P0025-06 [I]; A61P0025-28 [I]; A61P0027-02 [I]; A61P0029-00 [I]; A61P0031-04 [I]; A61P0031-14 [I]; A61P0031-18 [I]; A61P0035-00 [I]; A61P0035-04 [I]; A61P0037-02 [I]; A61P0037-06 [I]; A61P0043-00 [I]; C07F0009-09 [I]; C07F0009-38 [I]
EXF 558/70; 558/166; 558/169; 514/114
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 254 OF 256 USPAT2 on STN
Full Text
AN 2002:158063 USPAT2
TI Intravascular delivery of methylprednisolone
IN Sirhan, Motasim, Sunnyvale, CA, UNITED STATES
Yan, John, Los Gatos, CA, UNITED STATES
PA Avantec Vascular Corporation, Sunnyvale, CA, UNITED STATES (U.S. corporation)
PI US 7018405 B2 20060328
AI US 2001-782804 20010213 (9)
PRAI US 2000-258024P 20001222 (60)
DT Utility
FS GRANTED
LN.CNT 966

INCL INCLM: 623/001.420
INCLS: 623/001.450
NCL NCLM: 623/001.420; 623/001.150
NCLS: 623/001.450; 623/001.430
CPC CPCI A61F0002-90 [I]; A61F2250-0067; A61L0027-54 [I]; A61L0031-16 [I]; A61L2300-41; A61L2300-416; A61L2300-602
CPCI-2 A61F0002-90 [I]; A61F2250-0067; A61L0027-54 [I]; A61L0031-16 [I]; A61L2300-41; A61L2300-416; A61L2300-602
IPC IPCI A61F0002-06 [ICM,7]
IPCI-2 A61F0002-06 [I]
IPCR A61F0002-06 [I]; A61F0002-00 [N]; A61F0002-90 [I]; A61L0027-54 [I]; A61L0031-16 [I]
EXF 623/1.42; 623/1.43; 623/1.44; 623/1.45; 623/1.46; 623/1.47; 623/1.48
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 255 OF 256 USPAT2 on STN
Full Text
AN 2002:141646 USPAT2
TI Production of 2-amin-2-[2-(2-(40-C2-20-alkyl-phenyl)ethyl]propane-1,3-diols
IN Abel, Stephan, Weil, GERMANY, FEDERAL REPUBLIC OF
Fujita, Tetsuro, Kyoto, JAPAN
Hirose, Ryoji, Hyogo, JAPAN
Jordine, Guido, Freiburg, GERMANY, FEDERAL REPUBLIC OF
Mishina, Tadashi, Osaka, JAPAN
PA Novartis AG, Basel, SWITZERLAND (non-U.S. corporation)
Taito Co. Ltd., Tokyo, JAPAN (non-U.S. corporation)
Mitsubishi Pharma Corp., Osaka, JAPAN (non-U.S. corporation)
PI US 6605744 B2 20030812
AI US 2001-850851 20010508 (9)
RLI Continuation of Ser. No. WO 1999-EP8577, filed on 9 Nov 1999
PRAI GB 1998-24705 19981111
GB 1998-24706 19981111
DT Utility
FS GRANTED
LN.CNT 743
INCL INCLM: 564/357.000
INCLS: 564/355.000; 564/356.000; 564/358.000
NCL NCLM: 564/357.000; 564/356.000
NCLS: 564/355.000; 564/356.000; 564/358.000
CPC CPCI C07C0233-47 [I]; C07C0017-16 [I]; C07C0017-208 [I]; C07C0017-35 [I]; C07C0022-04 [I]; C07C0029-00 [I]; C07C0045-46 [I]; C07C0045-63 [I]; C07C0213-00 [I]; C07C0215-28 [I]
CPCI-2 C07C0233-47 [I]; C07C0017-16 [I]; C07C0017-208 [I]; C07C0017-35 [I]; C07C0022-04 [I]; C07C0029-00 [I]; C07C0045-46 [I]; C07C0045-63 [I]; C07C0213-00 [I]; C07C0215-28 [I]
IPC [7]
IPCI C07C0215-56 [ICM,7]
IPCI-2 C07C0209-24 [ICM,7]
IPCR C07C0017-16 [I]; C07C0017-20 [I]; C07C0017-35 [I]; C07C0022-04 [I]; C07C0029-00 [I]; C07C0045-46 [I]; C07C0045-63 [I]; C07C0213-00 [I]; C07C0213-02 [I]; C07C0215-28 [N]; C07C0215-34 [I]; C07C0231-12 [I]; C07C0233-05 [I]; C07C0233-47 [I]
EXF 564/356; 564/355; 564/357; 564/358
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 256 OF 256 USPAT2 on STN
Full Text
AN 2001:114679 USPAT2
TI Process for preparing 2-amino malonic acid derivatives and 2-amino-1,3-propanediol derivatives, and intermediates for preparing the same
IN Hirase, Susumu, Kobe, Japan
Sasaki, Shigeo, Kobe, Japan
Yoneta, Masahiko, Hadano, Japan
Hirose, Ryoji, Kobe, Japan
Fujita, Tetsuro, Muko, Japan
PA Taito Co., LTD, Tokyo, Japan (non-U.S. corporation)
Welfide Corporation, Osaka, Japan (non-U.S. corporation)
PI US 6284915 B2 20010904
AI US 1999-261342 19990303 (9)
RLI Continuation of Ser. No. WO 1998-JP2998, filed on 3 Jul 1998

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PRAI   JP 1997-2299           19970703
DT     Utility
FS     GRANTED
LN.CNT 1061
INCL   INCLM: 560/039.000
       INCLS: 560/040.000; 568/031.000; 568/331.000; 568/336.000
NCL    NCLM:  560/039.000
       NCLS:  560/040.000; 568/031.000; 568/331.000; 568/336.000
CPC    CPCI   C07C0309-73 [I]; C07C0045-63 [I]; C07C0045-673 [I]; C07C0213-08
              [I]; C07C0227-16 [I]; C07C0229-36 [I]; C07C0233-47 [I]
       CPCI-2 C07C0309-73 [I]; C07C0045-63 [I]; C07C0045-673 [I]; C07C0213-08
              [I]; C07C0227-16 [I]; C07C0229-36 [I]; C07C0233-47 [I]
IPC    [7]
       IPCI   C07C0229-00 [ICM,7]
       IPCI-2 C07C0229-28 [ICM,7]
       IPCR   C07C0045-63 [I]; C07C0045-67 [I]; C07C0213-08 [I]; C07C0215-28
              [N]; C07C0227-16 [I]; C07C0229-36 [I]; C07C0233-47 [I];
              C07C0309-73 [I]
EXF    568/336; 568/331; 568/31; 560/39; 560/40
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


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                                                   ENTRY    SESSION
FULL ESTIMATED COST                                83.88      94.01

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13149468	HIESTAND ET AL.
	Examiner	Art Unit
	KEVIN E WEDDINGTON	1629

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
514	667	9/24/2013	KEW
514	903	9/24/2013	KEW

SEARCH NOTES		
Search Notes	Date	Examiner
CAS-ONLINE search with REGISTRY and USPATALL	9/24/2013	KEW
EAST and PALM for Inventors' Names for Related Applications for ODP	9/24/2013	KEW

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

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www.uspto.gov

BIB DATA SHEET

CONFIRMATION NO. 1536

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
13/149,468	05/31/2011 RULE	514	1629	PAT050279-US-CNT

APPLICANTS

Peter C. Hiestand, Allschwil, SWITZERLAND;
Christian Schnell, Hesingue, FRANCE;

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

This application is a CON of 12/303,765 12/08/2008 ABN
which is a 371 of PCT/EP2007/005597 06/25/2007

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

UNITED KINGDOM 0612721.1 06/27/2006

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

06/15/2011

Foreign Priority claimed	35 USC 119(a-d) conditions met	Verified and Acknowledged	Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
☑ Yes ☐ No	☑ Yes ☐ No	/KEVIN E WEDDINGTON/ Examiner's Signature	☐ Initials	SWITZERLAND	0	10	3

ADDRESS

NOVARTIS PHARMACEUTICAL CORPORATION
INTELLECTUAL PROPERTY DEPARTMENT
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080
UNITED STATES

TITLE

S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS

FILING FEE RECEIVED		
1480	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

PTO/SB/08a (07-09)
PTO/SB/08b (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	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[2]
		K. Rammohan et al, Poster on 'Long-Term Safety of Fingolimod in Patients with Relapsing-Remitting Multiple Sclerosis: Results from Phase 3 FREEDOMS II Extension Study' March 16-23 2013, San Diego, US, 65th American Academy of Neurology Annual Meeting.	☐
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Examiner Signature		**Date Considered**	

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

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 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 2 hours 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, 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:	15550410
Application Number:	13149468
International Application Number:	
Confirmation Number:	1536
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Customer Number:	1095
Filer:	Karen DeBenedictis/Denise Cooper
Filer Authorized By:	Karen DeBenedictis
Attorney Docket Number:	PAT050279-US-CNT
Receipt Date:	18-APR-2013
Filing Date:	31-MAY-2011
Time Stamp:	11:15:06
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		50279.pdf	78442 be0887518ee4346007557307dc58801c755f2643	yes	2

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Transmittal Letter	1	1
	Information Disclosure Statement (IDS) Form (SB08)	2	2

Warnings:					
Information:					
2	Non Patent Literature	Rammohan_FREEDOMS-II-Ext-Safety_AAN-2013March.pdf	995217 7d9033da33c5e6be22b087a12eb32017a654315a	no	1
Warnings:					
Information:					
		Total Files Size (in bytes):	1073659		

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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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO: 13/149468
FILED: May 31, 2011
FOR: DOSAGE REGIMEN OF AN S1P RECEPTOR AGONIST

Art Unit: 1629
Examiner:

MS: Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Sir:

This paper is being filed:

☒ before the mailing date of a first Office Action on the merits, and so under 37 C.F.R. §1.97(b)(3) no fees are required.

If a fee is deemed to be required, the Commissioner is hereby authorized to charge such fee to Deposit Account No. 19-0134 in the name of Novartis.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO/SB/08A/B.

☒ Copies of the references are enclosed herewith.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO/SB/08A/B form(s).

Respectfully submitted,

Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936
+1 862 7783785
Date: 4/17/13

PTO/SB/30 (07-09)
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Request for Continued Examination (RCE) Transmittal

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	1629
Examiner Name	SPIVACK, PHYLLIS G
Attorney Docket Number	PAT050279-US-CNT

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO) on page 2.

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant must request non-entry of such amendment(s).
   - a. ☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.
     - i. ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
     - ii. ☐ Other _____
   - b. ☒ Enclosed
     - i. ☒ Amendment/Reply
     - ii. ☐ Affidavit(s)/Declaration(s)
     - iii. ☐ Information Disclosure Statement (IDS)
     - iv. ☐ Other _____

2. Miscellaneous
   - a. ☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of _____ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)
   - b. ☐ Other _____

3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.
   - a. ☒ The Director is hereby authorized to charge the following fees, any underpayment of fees, or credit any overpayments, to Deposit Account No. 19-0134 in the name of Novartis.
     - i. ☒ RCE fee required under 37 CFR 1.17(e)
     - ii. ☐ Extension of time fee (37 CFR 1.136 and 1.17)
     - iii. ☐ Other _____
   - b. ☐ Check in the amount of $_____ enclosed
   - c. ☐ Payment by credit card (Form PTO-2038 enclosed)

WARNING: Information on this form may become public. Credit Card information should not be included on this form. Provide credit card information and authorization on PTO-2038

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature	/Karen DeBenedictis /	Date	February 18, 2013
Name (Print/Type)	Karen DeBenedictis	Registration No.	32,977

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 or facsimile transmitted to the U.S. Patent and Trademark Office on the date shown below.

Signature			
Name (Print/Type)		Date	

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. *et al.*
APPLICATION NO: 13/149468
FILED: May 31, 2011
FOR: DOSAGE REGIMEN OF AN S1P RECEPTOR AGONIST

Art Unit: 1629
Examiner: Spivack, Phyllis G.
Conf. No.: 1536

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

AMENDMENT

Sir:

This Amendment is being submitted with a Request for Continued Examination pursuant to 37 CFR §1.114 in response to the Office Action in the above application that was mailed to Applicants' attorney on November 16, 2012 (the "Office Action"). A response to the Office Action is due by February 16, 2013.

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

Remarks/Arguments begin on page 4 of this paper.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1 – 11. (Cancelled).

Claim 12. (Currently amended) A method for inhibiting or treating neo-angiogenesis associated with multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg, absent an immediately preceding loading dose regimen.

Claim 13. (Currently amended) A method for reducing or alleviating relapses in primary progressive multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg, absent an immediately preceding loading dose regimen.

Claim 14. (Currently amended) A method according to claim 12, wherein said multiple sclerosis is primary progressive ~~relapsing-remitting~~ multiple sclerosis. ~~for inhibiting or treating neo-angiogenesis associated with relapsing-remitting multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.~~

Claim 15. (Canceled)

Claim 16. (Currently amended) A method according to claim 12 ~~or 13~~ wherein 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride is administered.

Claim 17. (Currently amended) A method for slowing progression of multiple sclerosis in a subject in the relapsing-remitting phase of primary progressive multiple sclerosis ~~need thereof~~, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg, absent an immediately preceding loading dose regimen.

Claim 18. (Canceled)

Claim 19. (Canceled)

Claim 20. (Canceled)

Claim 21. (Previously presented) A method according to claim 13, wherein 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride is administered.

Claim 22. (New) A method according to claim 16, wherein said multiple sclerosis is primary-progressive multiple sclerosis.

Claim 23. (New) A method according to claim 14, wherein 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride is administered.

Claim 24. (New) A method according to claim 15, wherein 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride is administered.

Claim 25. (New) A method according to claim 12, wherein said multiple sclerosis is relapsing-remitting multiple sclerosis.

REMARKS

Reconsideration of the above application is respectfully requested.

There are 10 claims pending in this application. They are claims 12 – 21. By the above amendments, Applicants have amended claims 12 – 14, 16 – 17, and 21, canceled claims 15, 18 and 20 and added new claims 22 through 25. Claim 19 was canceled in a previously submitted paper. Applicants maintain their position that the subject matter deleted from the claims via the above amendments is patentable over the cited art, and have made such amendments to advance prosecution of the remaining subject matter. They reserve their right to prosecute the above deleted subject matter in subsequent divisional applications. These amendments add no new matter to the application.

In the Office Action, the Examiner maintained her rejection of claims 12 – 18 and 20 - 21 under 35 U.S.C. 103(a) as being unpatentable over Virley, D.J., Journal of the American Society for NeuroTherapeutics ("Virley"), in view of LaMontagne *et al.*, Cancer Research ("LaMontagne"), and further in view of Kovarik *et al.*, WO06/058316 ("Kovarik"), all of record. The Examiner stated that LaMontagne was included in the rejection merely to establish the utility of FTY720 as an anti-angiogenic agent. She further stated that Kovarik teaches a method for treating an autoimmune disease, in a subject in need thereof, with FTY720 at a daily dosage of about 0.1 to 0.5 mg, and that Virley teaches the administration of FTY720 for the treatment of multiple sclerosis. For the following reasons, Applicants traverse this rejection, as applied to all pending claims, as amended, and respectfully request that it be withdrawn.

Applicants have amended all pending claims (or the claims from which they depend) to specify that the stated daily dosage of 0.5 mg cannot immediately follow a loading dose regimen. Applicants have made these amendments to further distinguish their claims from the disclosure of Kovaric. Applicants have also amended the pending claims to specify that the disorder being treated by a daily dosage of 0.5 mg is either primary progressive multiple sclerosis (which includes the relapsing-remitting phase of primary progressive multiple sclerosis) or angiogenesis associated with multiple sclerosis.

Kovaric's mention of a dosage of 0.5 mg is limited to the context of a dosage that follows, immediately, the loading dose regimen described therein. The Kovaric loading dose regimen involves raising stepwise the daily dosage of the S1P receptor or agonist over 3 to 6 days up to 3- to 21-fold the standard daily dosage for the purpose of attaining steady state blood levels of the drug in less than a week. This loading dosage allows patients to continue, immediately following such regimen, with a daily dosage that is the same or lower than the standard daily dosage. Kovarik indicates that when the dosage administered after the initial period is lower than the standard daily dosage, it can be from 1/50 to ½ the standard daily dosage. Kovaric also states that the daily dosage employed following a loading dose regimen can be 0.1 – 0.5 mg or can be much larger – 2.5 or 5.0 mg. Thus, Kovaric teaches that the daily dosage

administered after the initial period can vary substantially relative to the standard daily dosage and is dependent on the immediately preceding loading dose administered during the initial phase. In addition to being only one of a wide range of dosages that can be employed following a loading dose regimen of an S1P receptor modulator or agonist, the 0.5 mg daily dosage referenced in Kovaric is mentioned only in the context of a dosage employed following the conclusion of a loading dose regimen. In view of the above, Applicants submit that the skilled person reading Kovaric would attach no significance to the suitability of any daily dosage mentioned therein outside the context of an immediately preceding loading dose regimen.

Kovarik makes no mention of angiogenesis, and no endpoints relating to angiogenesis were measured in connection with any dosage of FTY720. Also, Kovarik makes no distinction between primary progressive multiple sclerosis, relapsing-remitting multiple sclerosis and the relapsing-remitting phase of primary progressive multiple sclerosis. In view of this, Applicants submit that the skilled person reading Kovarik, as of the priority filing date of this application, would not have been motivated to inhibit or treat, with a reasonable expectation of success, angiogenesis associated with multiple sclerosis, or to treat, with a reasonable expectation of success, primary progressive multiple sclerosis with the low daily dosage of 0.5 mg FTY720.

Applicants further submit that neither Virley nor Lamontagne would provide the skilled person with any guidance regarding a favorable human dosage, *i.e.*, favorable with respect to the balance of safety and efficacy, to use for any of the disorders mentioned in Applicants' claims. They simply refer to the use of FTY720 to treat, respectively, multiple sclerosis and angiogenesis. As Applicants mentioned in the prior Amendment they submitted in this case, LaMontagne reports that dosages of 0.3 and 3.0 mg/kg FY720 were tested in an angiogenesis model in mice, and that the dosage of 0.3 mg/kg was less active in inhibiting angiogenesis than the dosage of 3.0 mg/kg. To the extent that this reference teaches anything about a favorable daily dosage of FTY720 for inhibiting angiogenesis, Applicants submit that it teaches away from the inventions of their pending claims, as amended. A dosage of 0.3 mg/kg corresponds to a dosage of 21.0 mg/day in an adult human weighing 70 kg, which is 42 times greater than the daily dosage of 0.5 mg/day that is a key feature of Applicants pending claims. Thus, Applicants respectfully submit that LaMontagne would not motivate the skilled person, and would not add anything to the disclosures of Kovaric and Lamontagne that would motivate the skilled person to treat angiogenesis associated with multiple sclerosis with a dosage of FTY720 as low as 0.5 mg, considering that the lower of the two FTY720 daily dosages mentioned in LaMontagne was stated therein to be not as effective as the higher of these two dosages, and the lower daily dosage was 42 times greater than 0.5 mg.

In view of the above, Applicants submit that all pending claims, as amended, comply fully

with 35 U.S.C. §103, and they respectfully request that such claims be allowed to issue.

Respectfully submitted,

/Karen DeBenedictis/

Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936
+1 8627783785

Date: 2/18/13

Electronic Patent Application Fee Transmittal

Application Number:	13149468
Filing Date:	31-May-2011
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Filer:	Karen DeBenedictis/Andrea Jacquin
Attorney Docket Number:	PAT050279-US-CNT

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

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

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Request for continued examination	1801	1	930	930
		Total in USD ($)		**930**

Electronic Acknowledgement Receipt

EFS ID:	14982503
Application Number:	13149468
International Application Number:	
Confirmation Number:	1536
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Customer Number:	1095
Filer:	Karen DeBenedictis/Andrea Jacquin
Filer Authorized By:	Karen DeBenedictis
Attorney Docket Number:	PAT050279-US-CNT
Receipt Date:	18-FEB-2013
Filing Date:	31-MAY-2011
Time Stamp:	16:02:25
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$930
RAM confirmation Number	11800
Deposit Account	190134
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		50279-US-CNT_RCE_Amendment_2013Feb18.pdf	2225762 05d8ff8d34b5f7138083956bd0954b24ed3e3e01	yes	7

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Request for Continued Examination (RCE)	1	1
Amendment After Final	2	2
Claims	3	4
Applicant Arguments/Remarks Made in an Amendment	5	7

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30488 82fd9434d2de45f3781928c84d4b3041dd5bcccf	no	2

Warnings:

Information:

Total Files Size (in bytes):	2256250

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

PTO/SB/06 (07-06)
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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
13/149,468	05/31/2011	

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	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT		(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 ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
	02/18/2013									
	Total (37 CFR 1.16(i))	* 10	Minus	** 20	= 0	X $ =		OR	X $62=	0
	Independent (37 CFR 1.16(h))	* 3	Minus	***3	= 0	X $ =		OR	X $250=	0
	☐ 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	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
	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 number found in the appropriate box in column 1.

Legal Instrument Examiner:
/Tina J. Barden/

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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/149,468	05/31/2011	Peter C. Hiestand	PAT050279-US-CNT	1536

1095 7590 11/16/2012

NOVARTIS PHARMACEUTICAL CORPORATION
INTELLECTUAL PROPERTY DEPARTMENT
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

EXAMINER	
SPIVACK, PHYLLIS G	
ART UNIT	**PAPER NUMBER**
1629	

NOTIFICATION DATE	DELIVERY MODE
11/16/2012	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):

php.patents@novartis.com

Office Action Summary	Application No.	Applicant(s)	
	13/149,468	HIESTAND ET AL.	
	Examiner	Art Unit	
	PHYLLIS G. SPIVACK	1629	

-- 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* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 July 2012*.

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) *12-18,20 and 21* 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) *12-18,20 and 21* 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 _____ 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).

a)☐ All b)☐ Some * c)☐ None of:

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/08) Paper No(s)/Mail Date *4/16/12; 7/19/12*.

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

4) ☐ Other: _____.

Applicants' Amendment filed July 27, 2012 is acknowledged. Claim 19 is canceled. Claims 12-18, 20 and 21 remain under consideration.

Information Disclosure Statements (IDS) filed April 16, 2012 and July 19, 2012 are further acknowledged and have been reviewed to the extent each reference is provided. It is noted the actual document RU2199339C2, cited on the IDS filed April 16, 2012, has not been supplied.

A new Abstract and an amendment to page 12 of the specification are noted.

The objections set forth in the last Office Action are withdrawn. The following rejection constitutes the only rejection presently applied to the present claims.

In the last Office Action claims12-21 were rejected under 35 U.S.C. 103(a) as being unpatentable over Virley, D.J., Journal of the American Society for Experimental NeuroTherapeutics, in view of LaMontagne et al., Cancer Research, and further in view of Kovarik et al., WO 06/058316. It was asserted Virley teaches the administration of 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-propane-1,3-diol, also known as FTY720, which is a sphingosine-1-phosphate receptor modulator, for the treatment of multiple sclerosis (MS). Virley distinguishes between the categories of relapsing-remitting MS and primary progressive MS. See the introduction on page 638, as well as the discussion of experimental models for MS on page 640. In order to provide predictive

indices for clinical application, experimental autoimmune encephalomyelitis (EAE) models are discussed. LaMontagne teaches FTY720 to be an anti-angiogenic agent. The compound becomes phosphorylated *in vivo* and interacts with spingosine-1-phosphate (S1P) receptors. The effect is on vascular permeability, an important aspect of angiogenesis. See the Abstract. Kovarik teaches dosage regimens involving S1P receptor agonists, of which FTY720 is clearly encompassed. On line 16, page 17, a daily dose of 0.5 mg is taught for the treatment of autoimmune diseases, of which MS is recited as an example, on lines 7-8 on page 14.

Applicants state FTY720 is efficacious for the treatment of multiple sclerosis as a fact, on page 6 of their Amendment. With respect to the LaMontagne reference, Applicants' argument is entirely directed to a dosage range disclosed therein for inhibiting angiogenesis. Applicants argue Kovarik makes no distinction among various immune disorders with respect to the dosages therein disclosed.

Applicants' arguments have been given careful consideration but are not found persuasive. The rejection of claims 12-18, 20 and 21 under 35 U.S.C. 103(a) as being unpatentable over Virley, D.J., Journal of the American Society for Experimental NeuroTherapeutics, in view of LaMontagne et al., Cancer Research, and further in view of Kovarik et al., WO 06/058316, is maintained. LaMontagne was included in the rejection of record merely to establish the utility of FTY720 as an anti-angiogenic agent. On page 17 in paragraph 2.5, Kovarik teaches a method for treating an autoimmune

disease in a subject in need thereof, wherein a daily dosage of FTY720 of about 0.1 to 0.5 mg is disclosed. With such guidance, through no more than routine experimentation, one skilled in the immunology art would have readily determined an optimal dosage range for a subject suffering from multiple sclerosis. See *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955) and MPEP §2144.05(II). The medical arts recognize that drug therapy may be optimized by designing regimens that account for the concentration of a drug, for example, to achieve a desired pharmacological response. Factors such as weight, age, gender, renal and hepatic status, *inter alia*, are always considered. Therefore, the determination of the optimum dosing regimen and dosage amounts would have been matters well within the purview of one of ordinary skill in the art, at the time of the invention, through no more than routine experimentation.

In view of the teachings of Virley, LaMontagne and Kovarik, one skilled in the neurology art would have been motivated to administer FTY720 with a reasonable expectation of success in inhibiting neo-angiogenesis associated multiple sclerosis, in alleviating relapses in MS and slowing the progression of MS. Such would have been obvious because FTY720 is a known anti-angiogenic agent that has been taught for use in the treatment of autoimmune diseases such as multiple sclerosis. A daily dosage of 0.5 mg is suggested by the prior art.

No claim is allowed.

THIS ACTION IS MADE FINAL. Applicants are reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this Final Action is set to expire THREE MONTHS from the mailing date of this Action. In the event a first reply is filed within TWO MONTHS of the mailing date of this Final Action and the Advisory Action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the Advisory Action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the Advisory Action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this Final Action.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Phyllis G. Spivack whose telephone number is 571-272-0585. The Examiner can normally be reached from 10:30 to 7 PM.

If attempts to reach the Examiner by telephone are unsuccessful after one business day, the Examiner's supervisor, Jeff Lundgren, can be reached 571-272-5541. 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).

November 13, 2012

/Phyllis G. Spivack/
Primary Examiner, Art Unit 1629

Receipt date: 04/16/2012

PTO/SB/08a (07-09)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al
Art unit	1629
Examiner Name	Spivack
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
		US-			
		US-			
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
		RU2199339C2	02-27-2003	TEKH; OOO BIO		☐
						☐
						☐
						☐
						☐
						☐

Examiner Signature	/Phyllis Spivack/	**Date Considered**	11/13/2012

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw a line through citation if not in conformance and not considered. Include copy of this form with the next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kind Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

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 2 hours 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, 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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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	1629
Examiner Name	Spivack
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
/P.S./		WO 2006/066086	06-22-2006	GENENTECH, INC.		☐
/P.S./		WO 2006/055809	05-26-2006	INCLONE SYSTEMS INCORPORATED		☐
						☐
						☐
						☐
						☐

Examiner Signature	/Phyllis Spivack/	**Date Considered**	11/13/2012

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw a line through citation if not in conformance and not considered. Include copy of this form with the next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kind Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

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 2 hours 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, 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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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	2	of	2

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	1629
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	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[2]
/P.S./		Fujino et al. 'Amelioration of experimental autoimmune encephalomyelitis...' The Journal of Pharmacology and Experimental Therapeutics, vol. 305, No.1, pp70-77. 2003.	☐
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Examiner Signature	/Phyllis Spivack/	**Date Considered**	11/13/2012

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

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF	Art Unit: 1629
Hiestand, Peter C. et al.	Examiner:
APPLICATION NO: 13/149468	Conf. No.:
FILED: May 31, 2011	
FOR: DOSAGE REGIMEN OF AN S1P RECEPTOR AGONIST	

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

AMENDMENT

Sir:

This Amendment is being submitted in response to the Office Action in the above application that was mailed to Applicants' attorney on May 3, 2012 ("Office Action") a response to which is due by August 3, 2012.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of the claims which begins on page 3 of this paper.

Remarks/Arguments begin on page 5 of this paper.

Amendments to the Specification:

On page 12, delete lines 22-23 and replace with:

--In particular, the S1P receptor modulators as described herein, e.g. FTY720, i.e. 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol, are useful for treating PP-MS.--

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1 – 11. (Cancelled).

Claim 12. (Previously presented) A method for inhibiting or treating neo-angiogenesis associated with multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 13. (Previously presented) A method for reducing or alleviating relapses in multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 14. (Previously presented) A method according to claim 12 for inhibiting or treating neo-angiogenesis associated with relapsing-remitting multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 15. (Previously presented) A method according to claim 13 for reducing or alleviating relapses in relapsing-remitting multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 16. (Previously presented) A method according to claim 12 or 13 wherein 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride is administered.

Claim 17. (Previously presented) A method for slowing progression of multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 18. (Previously presented) A method according to claim 17 wherein the disease is relapsing-remitting multiple sclerosis.

Claim 19. (Cancelled)

Claim 20. (Previously presented) A method according to claim 17 wherein the disease is primary-progressive multiple sclerosis (PP-MS).

Claim 21. (Previously presented) A method according to claim 17, wherein 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride is administered.

REMARKS

Reconsideration of the above application is respectfully requested. There are 10 claims pending in this application. They are claims 12 – 21. By the above amendments, Applicants have amended the specification to correct a spelling error noted by the Examiner on page 12 and have cancelled claim 19. These amendments add no new matter to the application.

In the Office Action, the Examiner objected to the Abstract for being broader than the scope of claimed subject matter under consideration. Applicants have attached to this paper a replacement Abstract, which they submit corresponds to the claimed subject matter being prosecuted in this application.

The Examiner also objected to the disclosure because of a spelling error on page 12. By the above amendments, Applicants have corrected the spelling of the misspelled chemical name.

In the Office Action, the Examiner rejected claims 12 - 21 under 35 U.S.C. 103(a) as being unpatentable over Virley, D.J., Journal of the American Society for NeuroTherapeutics ("Virley"), in view of LaMontagne *et al.*, Cancer Research ("LaMontagne"), and further in view of Kovarik *et al.*, WO06/058316 ("Kovarik"), all of record. The Examiner took the position that, in view of Virley's disclosure of the use of 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol ("FTY720") to treat multiple sclerosis, LaMontagne's reference to FTY720 as an anti-angiogenic agent and Kovarik's reference to the treatment of an autoimmuine disease such as multiple sclerosis with FY720 at a daily dosage of 0.5 mg, the skilled person would have been motivated, as of the priority filing date of this application, to administer FTY720 at a daily dosage of 0.5 mg for inhibiting the neo-angiogenesis associated with multiple sclerosis with a reasonable expectation of success. For the following reasons, Applicants traverse this rejection, as applied to the pending claims, as amended, and respectfully request that it be withdrawn.

Applicants submit that Kovarik would not suggest anything to the skilled person regarding the daily dosage that would be efficacious or advantageous for the treatment or inhibition of angiogenesis associated with multiple sclerosis. Kovarik refers to a loading dose regimen for FTY720 that is aimed at reaching a steady state blood level of the drug in less than a week. This is accomplished by administering the S1P receptor modulator or agonist during the initial 3 – 6 days, preferably, the initial 4 days of treatment, at a daily dosage that is higher than the standard daily dosage (*i.e.*, the dosage necessary for a steady-state trough blood level of the drug providing effective treatment) and is preferably increased stepwise during such initial period, after which the drug is administered at a dosage that is less than or equal to the standard daily dosage. Kovarik indicates that when the dosage administered after the initial period is lower than the standard daily dosage, it can be from 1/50 and ½ the standard daily dosage. Thus, the dosage administered after the initial period can vary substantially relative to the standard daily dosage and is also dependent on the immediately preceding loading dose

administered during the initial phase. The reference to a daily dosage of *0.5 mg* FTY720 on page 19 of Kovarik refers to a dosage that could be administered on day 5 of a treatment regimen following 4 days of administering the drug in accordance with the loading dose regimen set forth on page 18. Kovarik also states, on page 18, that the same loading dose regimen during days 1 – 4 can be followed by the administration of a daily dosage of *5.0 mg* FTY720. Kovarik makes no mention of angiogenesis, and no endpoints relating to angiogenesis were measured in connection with any dosage of FTY720. Also, Kovarik makes no distinction between different immune disorders with respect to any of the dosages mentioned therein. In view of this, Applicants submit that the skilled person reading Kovarik, as of the priority filing date of this application, would not be motivated to inhibit or treat the angiogenesis associated with multiple sclerosis, or any other immune disorder or symptom thereof, with a daily dosage of 0.5 mg FTY720.

Applicants wish to draw the Examiner's attention to another reason that Kovarik would not suggest to the skilled person any efficacious or advantageous dosage for any particular immune disorder or symptom thereof. The skilled person would not expect the optimal dosage or dosing regimen for treating transplant patients to be indicative of the optimal dosage or dosing regimen for treating an immune disorder such as multiple sclerosis. When treating a transplant patient, it is important to deliver a very high dose very early in treatment to counter rejection of the transplanted tissue, a consideration that does not apply to chronic immune disorders such as multiple sclerosis, where the goal is to degrade the disease over the period of the patient's natural life. Therefore, Kovarik's general disclosure of dosing regimen's suitable for transplant patients and for any other immune disorder would not suggest to the skilled person any particular dosage for any particular disorder or symptom thereof.

The main reference cited by the Examiner, Virley, provides no teaching relevant to Applicants' pending claims other than the fact that FTY720 is efficacious for the treatment of multiple sclerosis. For the reasons stated above, Kovarik adds nothing to the teachings of Virley that would suggest to the skilled person the efficacy of FTY720 in treating or inhibiting angiogenesis associated with multiple sclerosis, let alone a particular dosage for doing so.

Applicants submit that LaMontagne fails to provide the teaching that, taken together with with the teachings of Virley and Kovarik, would suggest the subject matter of Applicants' claims to the skilled person, specifically the treatment or inhibition of angiogenesis associated with multiple sclerosis by administering FTY720 at a daily dosage of 0.5 mg. Lamontagne reports that dosages of 0.3 and 3.0 mg/kg FY720 were tested in an angiogenesis model in mice, and that the dosage of 0.3 mg/kg was less active in inhibiting angiogenesis than the dosage of 3.0 mg/kg. However, a dosage of 0.3 mg/kg corresponds to a dosage of 21.0 mg/day in an adult human weighing 70 kg, which is 42 times greater than the daily dosage of 0.5 mg/day that is a feature of Applicants pending claims. Thus, Applicants respectfully submit that LaMontagne would not motivate the skilled person to treat angiogenesis associated with multiple sclerosis

with a dosage of FTY720 as low as 0.5 mg, considering that the lower of the two FTY720 daily dosages mentioned in LaMontagne was stated therein to be not as effective as the higher of these two dosages, and the lower daily dosage was 42 times greater than 0.5 mg.

In view of the above, Applicants submit that all pending claims, as amended, comply fully with 35 U.S.C. §103, and they respectfully request that such claims be allowed to issue.

Respectfully submitted,

Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

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Date: 7/26/12

Abstract

The present invention relates to the use of the S1P receptor modulator 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol, administered at a daily dosage of 0.5 mg, for inhibiting or treating neo-angiogenesis associated with multiple sclerosis.

Electronic Acknowledgement Receipt

EFS ID:	13355040
Application Number:	13149468
International Application Number:	
Confirmation Number:	1536
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Customer Number:	1095
Filer:	Karen DeBenedictis/Denise Cooper
Filer Authorized By:	Karen DeBenedictis
Attorney Docket Number:	PAT050279-US-CNT
Receipt Date:	27-JUL-2012
Filing Date:	31-MAY-2011
Time Stamp:	09:41:39
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		50279-US-CNT-Amendment.pdf	252323 2b3a53b9ddb18aef4413e46d06636e9d36294fda	yes	8

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	7
	Abstract	8	8
Warnings:			
Information:			
	Total Files Size (in bytes):	252323	

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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.

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 07/31/2012

MBLYTHER SALE #00000001 Mailroom Dt: 07/27/2012 190134 13149468
01 FC : 1251 150.00 DA

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
13/149,468	05/31/2011	

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	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	07/27/2012	(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 ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 8	Minus	** 20	= 0	X $ =		OR	X $60=	0
	Independent (37 CFR 1.16(h))	* 3	Minus	***3	= 0	X $ =		OR	X $250=	0
	☐ 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	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
	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 number found in the appropriate box in column 1.

Legal Instrument Examiner:
/MARISSA BLYTHER/

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	2

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	1629
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
		WO 2006/066086	06-22-2006	GENENTECH, INC.		☐
		WO 2006/055809	05-26-2006	INCLONE SYSTEMS INCORPORATED		☐
						☐
						☐
						☐
						☐

Examiner Signature		**Date Considered**	

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PTO/SB/08b (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	1629
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	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[2]
		Fujino et al. 'Amelioration of experimental autoimmune encephalomyelitis...' The Journal of Pharmacology and Experimental Therapeutics, vol. 305, No.1, pp70-77.	☐
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Examiner Signature		**Date Considered**	

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[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.
This collection of information is required by 37 CFR 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 2 hours 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, 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 Patent Application Fee Transmittal

Application Number:	13149468
Filing Date:	31-May-2011
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Filer:	Karen DeBenedictis/Denise Cooper
Attorney Docket Number:	PAT050279-US-CNT

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
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:	13296073
Application Number:	13149468
International Application Number:	
Confirmation Number:	1536
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Customer Number:	1095
Filer:	Karen DeBenedictis/Denise Cooper
Filer Authorized By:	Karen DeBenedictis
Attorney Docket Number:	PAT050279-US-CNT
Receipt Date:	19-JUL-2012
Filing Date:	31-MAY-2011
Time Stamp:	17:39:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$180
RAM confirmation Number	4606
Deposit Account	190134
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Foreign Reference	WO06066086.pdf	2577033 12b263e72156c59049521a02c0da37d90d855719	no	47
Warnings:					
Information:					
2	Foreign Reference	WO06055809.pdf	3995924 908df983b761068e237494f1d13159a63353000c	no	82
Warnings:					
Information:					
3		50279-US-CNT-IDS.pdf	146155 46477c95338cd90dd304a9151a8709bd613eafc1	yes	3

Multipart Description/PDF files in .zip description

Document Description	Start	End
Transmittal Letter	1	1
Information Disclosure Statement (IDS) Form (SB08)	2	3

Warnings:					
Information:					
4	Non Patent Literature	Fujino.pdf	501344 7c7844805f629443ef5a30b6be276a807f419240	no	8
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30482 ab18586e9bdc12ab08dcd55ace2263119f4241d7	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	7250938		

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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.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 June 2006 (22.06.2006)

PCT

(10) International Publication Number
WO 2006/066086 A1

(51) International Patent Classification:
C07K 16/22 (2006.01) *A61K 39/395* (2006.01)

(21) International Application Number:
PCT/US2005/045600

(22) International Filing Date:
16 December 2005 (16.12.2005)

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(54) **Title:** ANTIANGIOGENESIS THERAPY OF AUTOIMMUNE DISEASE IN PATIENTS WHO HAVE FAILED PRIOR THERAPY

(57) **Abstract:** The present application describes therapy with angiogenesis antagonists such as anti-VEGF antibodies. In particular, the application describes the use of such antagonists to treat autoimmune disease in a patient who has failed prior treatment such as treatment with DMARDs or TNFα-inhibitors.

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ANTIANGIOGENESIS THERAPY OF AUTOIMMUNE DISEASE IN PATIENTS WHO HAVE FAILED PRIOR THERAPY

Related Applications

This is a non-provisional application filed under 37 CFR §1.53(b), claiming priority under 35 U.S.C. §119(e) to U.S. Provisional Application Serial No. 60/637,169 filed on December 17, 2004, the entire contents of which is hereby incorporated by reference.

Field of the Invention

The present invention concerns therapy with angiogenesis antagonists, such as an anti-VEGF antibody. In particular, the invention concerns the use of such antagonists to treat autoimmune disease, particularly in a patient who has failed prior treatment.

Background of the Invention

Autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, vasculitis, and lupus, among others, remain clinically important diseases in humans. Collectively, autoimmune diseases affect about 5% of North Americans and Europeans, two-thirds of whom are women. As the name implies, autoimmune diseases wreak their havoc through the body's own immune system. The immune system, normally efficient in defeating external threats from the microbial world, at times directs its potent arsenal against the body's self-constituents, causing autoimmunity. While the pathological mechanisms differ among individual types of autoimmune diseases, one general mechanism involves the binding of certain antibodies (referred to herein as self-reactive antibodies or autoantibodies) present. The diseases often involve distinct anatomic regions. For example, the immune system attacks the synovial lining of the joints in rheumatoid arthritis (RA), the thyroid gland in thyroiditis, the insulin-secreting beta cells of the pancreas in type 1 diabetes mellitus (T1DM), and the myelin sheath of the brain and the spinal cord in multiple sclerosis (MS). In systemic lupus erythematosus (SLE), there are protean manifestations with involvement of skin, kidneys, joints, and brain.

Rheumatoid arthritis (RA) is a chronic autoimmune disorder of unknown etiology, typically characterized by symmetrical pain and swelling of the small joints of the hands and feet. Virtually any other joint in the body may become affected by inflammation, including the large joints, such as the shoulders, knees, and hips, jaws, and cervical spine. Persistent joint inflammation often produces articular cartilage and bone destruction as well as permanent deformities. The natural history of disease is described in years, but joint damage may occur as early as 3 to 6 months after onset. Although RA predominantly affects the joints, it is a systemic disease and may cause fatigue, low-grade fever, and involve other organ systems, including the eyes, lungs, and blood vessels. For example, RA may cause scleritis

(inflammatory eye disease), pleuritis, interstitial pulmonary fibrosis, and vasculitis. RA exacts a considerable toll on a patient's quality of life, causing pain and functional disability, with associated restrictions on household, family, and recreational activities. Limitations in work capacity and in some cases, unemployment, can have substantial economic ramifications for both individuals and society.

The diagnosis of RA is based on clinical manifestations and the results of selected laboratory tests. Approximately 75% of patients will test positive for rheumatoid factor (an autoantibody reactive with the Fc portion of immunoglobulin G [IgG]), but this finding may not be present during the first year of disease. Furthermore, rheumatoid factor is not specific for rheumatoid arthritis and is found in 5% of healthy individuals. The erythrocyte sedimentation rate is increased in most patients with RA, and C-reactive protein, another acute phase reactant, is typically elevated in patients with active disease. X-rays of the hands and feet, or possibly other joints, may be useful in some cases, demonstrating periarticular bony demineralization, joint space narrowing, and bony erosions.

Currently there is no cure for RA. Since the cause of the disease is unknown, current therapies are directed toward suppression of the inflammatory response. Like most chronic arthritides, the goal of treatment is to preserve joint function and limit disease progression. The medication list of a patient with active RA may include a nonsteroidal anti-inflammatory drug (NSAID), a low dose of prednisone, and one or more disease-modifying antirheumatic drugs (DMARDs). See "Guidelines for the management of rheumatoid arthritis" *Arthritis & Rheumatism* 46(2): 328-346 (February, 2002). The majority of patients with newly diagnosed RA are started with disease-modifying antirheumatic drug (DMARD) therapy within 3 months of diagnosis. DMARDs commonly used in RA are hydroxycloroquine, sulfasalazine, methotrexate (MTX), leflunomide, azathioprine, D-penicillamine, Gold (oral), Gold (intramuscular), minocycline, cyclosporine, and Staphylococcal protein A immunoadsorption. Recent studies indicate that patients with active RA develop significant joint damage during the first few years of disease. This knowledge has led to a more aggressive treatment approach using combinations of DMARDs. However, combination DMARD therapy does not completely abrogate disease activity and may result in serious drug-related complications. Moreover, most patients still develop joint erosions despite aggressive treatment.

Overactivity of the cytokine tumor necrosis factor (TNF) has been associated with synoviocyte proliferation, neo-angiogenesis, the recruitment of inflammatory cells, and the production of degradative enzymes. These findings have stimulated the development of anticytokine therapies. Further investigation has shown that the signs and symptoms of RA can be abrogated when certain proinflammatory cytokines, such as TNF and IL-1, are neutralized by monoclonal antibodies, naturally occurring cytokine antagonists, or cytokine receptor blockers.

Etanercept (ENBREL®) is an injectable drug approved in the US for therapy of active RA. Etanercept binds to TNFα and serves to remove most TNFα from joints and blood, thereby preventing TNFα from promoting inflammation and other symptoms of rheumatoid arthritis. Etanercept is an "immunoadhesin" fusion protein consisting of the extracellular ligand binding portion of the human 75 kD (p75) tumor necrosis factor receptor (TNFR) linked to the Fc portion of a human IgG1. The drug has been associated with negative side effects including serious infections and sepsis, nervous system disorders such as multiple sclerosis (MS).

Infliximab, sold under the trade name REMICADE®, is an immune-suppressing drug prescribed to treat RA and Crohn's disease. Infliximab is a chimeric monoclonal antibody that binds to TNFα and reduces inflammation in the body by targeting and binding to TNFα which produces inflammation. Infliximab has been linked to fatal reactions such as heart failure and infections including tuberculosis as well as demyelination resulting in MS.

In December 2002, Abbott Laboratories received FDA approval to market adalimumab (HUMIRA™), previously known as D2E7. Adalimumab is a human monoclonal antibody that binds to TNFα and is approved for reducing the signs and symptoms and inhibiting the progression of structural damage in adults with moderately to severely active RA who have had insufficient response to one or more traditional disease modifying DMARDs.

Angiogenesis is an important cellular event in which vascular endothelial cells proliferate, prune and reorganize to form new vessels from preexisting vascular network. There are compelling evidences that the development of a vascular supply is essential for normal and pathological proliferative processes (Folkman and Klagsbrun (1987) *Science* 235:442-447). Delivery of oxygen and nutrients, as well as the removal of catabolic products, represent rate-limiting steps in the majority of growth processes occurring in multicellular organisms. Thus, it has been generally assumed that the vascular compartment is necessary, albeit but not sufficient, not only for organ development and differentiation during embryogenesis, but also for wound healing and reproductive functions in the adult.

Angiogenesis is also implicated in the pathogenesis of a variety of disorders, including but not limited to, proliferative retinopathies, age-related macular degeneration, tumors, autoimmune diseases such as rheumatoid arthritis (RA), and psoriasis. Angiogenesis is a cascade of process consisting of 1) degradation of the extracellular matrix of a local venue after the release of protease, 2) proliferation of capillary endothelial cells, and 3) migration of capillary tubules toward the angiogenic stimulus. Ferrara et al. (1992) *Endocrine Rev.* 13:18-32.

In view of the remarkable physiological and pathological importance of angiogenesis, much work has been dedicated to the elucidation of the factors capable of regulating this

process. It is suggested that the angiogenesis process is regulated by a balance between pro- and anti- angiogenic molecules, and is derailed in various diseases, especially cancer. Carmeliet and Jain (2000) Nature 407:249-257.

Vascular endothelial cell growth factor (VEGF), a potent mitogen for vascular endothelial cells, has been reported as a pivotal regulator of both normal and abnormal angiogenesis. Ferrara and Davis-Smyth (1997) *Endocrine Rev.* 18:4-25; Ferrara (1999) J. Mol. Med. 77:527-543. Compared to other growth factors that contribute to the processes of vascular formation, VEGF is unique in its high specificity for endothelial cells within the vascular system. Recent evidence indicates that VEGF is essential for embryonic vasculogenesis and angiogenesis. Carmeliet et al. (1996) *Nature* 380:435-439; Ferrara et al. (1996) *Nature* 380:439-442. Furthermore, VEGF is required for the cyclical blood vessel proliferation in the female reproductive tract and for bone growth and cartilage formation. Ferrara et al. (1998) *Nature Med.* 4:336-340; Gerber et al. (1999) *Nature* Med. 5:623-628.

In addition to being an angiogenic factor in angiogenesis and vasculogenesis, VEGF, as a pleiotropic growth factor, exhibits multiple biological effects in other physiological processes, such as endothelial cell survival, vessel permeability and vasodilation, monocyte chemotaxis and calcium influx. Ferrara and Davis-Smyth (1997), *supra*. Moreover, recent studies have reported mitogenic effects of VEGF on a few non-endothelial cell types, such as retinal pigment epithelial cells, pancreatic duct cells and Schwann cells. Guerrin et al. (1995) J. Cell Physiol. 164:385-394; Oberg-Welsh et al. (1997) Mol. Cell. Endocrinol. 126:125-132; Sondell et al. (1999) J. Neurosci. 19:5731-5740.

Substantial evidence also implicates VEGF's critical role in the development of conditions or diseases that involve pathological angiogenesis. The VEGF mRNA is overexpressed by the majority of human tumors examined (Berkman *et al. J Clin Invest* 91:153-159 (1993); Brown *et al. Human Pathol.*. 26:86-91 (1995); Brown *et al. Cancer Res.* 53:4727-4735 (1993); Mattern *et al. Brit. J. Cancer.* 73:931-934 (1996); and Dvorak *et al. Am J. Pathol.* 146:1029-1039 (1995)). Also, the concentration of VEGF in eye fluids are highly correlated to the presence of active proliferation of blood vessels in patients with diabetic and other ischemia-related retinopathies (Aiello *et al. N. Engl. J. Med.* 331:1480-1487 (1994)). Furthermore, recent studies have demonstrated the localization of VEGF in choroidal neovascular membranes in patients affected by AMD (Lopez *et al. Invest. Ophtalmo. Vis. Sci.* 37:855-868 (1996)).

The recognition of VEGF as a primary regulator of angiogenesis in pathological conditions has led to numerous attempts to block VEGF activities. Inhibitory anti-VEGF receptor antibodies, soluble receptor constructs, antisense strategies, RNA aptamers against VEGF and low molecular weight VEGF receptor tyrosine kinase (RTK) inhibitors have all been proposed for use in interfering with VEGF signaling (Siemeister et al. *Cancer*

Metastasis Rev. 17:241-248 (1998). Indeed, anti-VEGF neutralizing antibodies have been shown to suppress the growth of a variety of human tumor cell lines in nude mice (Kim *et al. Nature* 362:841-844 (1993); Warren *et al. J. Clin. Invest.* 95:1789-1797 (1995); Borgström *et al. Cancer Res.* 56:4032-4039 (1996); and Melnyk *et al. Cancer Res.* 56:921-924 (1996)) and also inhibit intraocular angiogenesis in models of ischemic retinal disorders (Adamis *et al. Arch. Ophthalmol.* 114:66-71 (1996)). Therefore, anti-VEGF monoclonal antibodies or other inhibitors of VEGF action are promising candidates for the treatment of solid tumors and various intraocular neovascular disorders. Although the VEGF molecule is upregulated in tumor cells, and its receptors are upregulated in tumor infiltrated vascular endothelial cells, the expression of VEGF and its receptors remain low in normal cells that are not associated with angiogenesis. Thus, such normal cells would not be affected by blocking the interaction between VEGF and its receptors to inhibit tumor angiogenesis, and therefore tumor growth and cancer metastasis.

Monoclonal antibodies are now commonly manufactured using recombinant DNA technology. Widespread use has been made of monoclonal antibodies, particularly those derived from rodents. However, nonhuman antibodies are frequently antigenic in humans. The art has attempted to overcome this problem by constructing "chimeric" antibodies in which a nonhuman antigen-binding domain is coupled to a human constant domain (Cabilly et al., U.S. patent No. 4,816,567). The isotype of the human constant domain may be selected to tailor the chimeric antibody for participation in antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity. In a further effort to resolve the antigen binding functions of antibodies and to minimize the use of heterologous sequences in human antibodies, humanized antibodies have been generated for various antigens in which substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species have substituted rodent (CDR) residues for the corresponding segments of a human antibody to generate. In practice, humanized antibodies are typically human antibodies in which some complementarity determining region (CDR) residues and possibly some framework region (FR) residues are substituted by residues from analogous sites in rodent antibodies. Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-327 (1988); Verhoeyen et al., Science 239:1534-1536 (1988).

Several humanized anti-human VEGF (hVEGF) antibodies have been successfully generated, and have shown significant hVEGF-inhibitory activities both in vitro and in vivo. Presta et al. (1997) Cancer Research 57:4593-4599; Chen et al. (1999) J. Mol. Biol. 293:865-881. One specific humanized anti-VEGF antibody, bevacizumab (Avastin®, Genentech, Inc.), has been approved in the US for use in combination with chemotherapeutic agents for treating metastatic colorectal cancer (CRC). The drug is currently used in several clinical trials for

treating various other cancers. Another high-affinity variant of the humanized anti-VEGF antibody is currently clinically tested for treating age-related macular degeneration (AMD).

There is increasing evidence to suggest that VEGF is associated with the pathogenesis of inflammatory joint diseases such as RA. VEGF has been identified in synovial tissues such as synovial lining cells, synovial lining macrophages, perivascular fibroblasts, and vascular smooth muscle cells in the inflamed joints of patients with RA. Nagashima et al (1995) *J. Rheumatol.* 22:1624-1630. VEGF levels in synovial fluid and serum are found to be significantly elevated in both adult and juvenile RA and to correlate with disease activity. Koch et al. (1994) *J. Immunol.* 152:4149-4156. Recently, it has been demonstrated that neutralization of VEGF can prevent collagen-induced arthritis and ameliorate established RA in mice. Sone et al. (2001) *Bioch. Bioph. Res. Comm.* 281:562-568.

Despite these developments, there remains a need for effective therapies of autoimmune diseases, especially therapies using angiogenesis antagonists.

Summary of the Invention

The present invention provides, in a first aspect, a method of treating an autoimmune disease in a mammal who has failed a prior treatment, comprising administering to the mammal a therapeutically effective amount of an angiogenesis antagonist.

For instance, the invention provides a method of treating rheumatoid arthritis in a mammal who has failed or experiences an inadequate response to a DMARD therapy such as MTX or a TNFα-inhibitor, comprising administering to the mammal a therapeutically effective amount of an antibody that binds to and blocks VEGF.

The invention also concerns a method of reducing the risk of a negative side effect selected from the group consisting of an infection, heart failure and demyelination, comprising administering to a mammal with an autoimmune disease a therapeutically effective amount of an angiogenesis antagonist.

Also provided are uses of angiogenesis antagonists such as anti-VEGF antibodies in the preparation of medicaments for the treatment of autoimmune diseases such as RA, in patients who have failed prior therapies.

Detailed Description of the Preferred Embodiments

I. Definitions

For the purposes herein, "angiogenesis antagonist" is a composition capable of blocking, inhibiting, abrogating, interfering or reducing pathological angiogenesis associated with a disease or disorder. Many angiogenesis antagonists have been identified and are known in the arts, including those listed by Carmeliet and Jain (2000). Generally, angiogenesis antagonist is a composition targeting a specific angiogenic factor or an angiogenesis pathway. In certain aspects, the angiogenesis antagonist is a protein

composition such as an antibody targeting an angiogenic factor. One of the most recognized angiogenic factors is VEGF, and one of the most potent angiogenesis antagonists is a neutralizing anti-VEGF antibody.

The terms "VEGF" and "VEGF-A" are used interchangeably to refer to the 165-amino acid vascular endothelial cell growth factor and related 121-, 189-, and 206- amino acid vascular endothelial cell growth factors, as described by Leung *et al. Science*, 246:1306 (1989), and Houck *et al. Mol. Endocrin.*, 5:1806 (1991), together with the naturally occurring allelic and processed forms thereof. The term "VEGF" is also used to refer to truncated forms of the polypeptide comprising amino acids 8 to 109 or 1 to 109 of the 165-amino acid human vascular endothelial cell growth factor. Reference to any such forms of VEGF may be identified in the present application, e.g., by "VEGF (8-109)," "VEGF (1-109)" or "$VEGF_{165}$." The amino acid positions for a "truncated" native VEGF are numbered as indicated in the native VEGF sequence. For example, amino acid position 17 (methionine) in truncated native VEGF is also position 17 (methionine) in native VEGF. The truncated native VEGF has binding affinity for the KDR and Flt-1 receptors comparable to native VEGF.

An "anti-VEGF antibody" is an antibody that binds to VEGF with sufficient affinity and specificity. Preferably, the anti-VEGF antibody of the invention can be used as a therapeutic agent in targeting and interfering with diseases or conditions wherein the VEGF activity is involved. An anti-VEGF antibody will usually not bind to other VEGF homologues such as VEGF-B or VEGF-C, nor other growth factors such as PlGF, PDGF or bFGF. A preferred anti-VEGF antibody is a monoclonal antibody that binds to the same epitope as the monoclonal anti-VEGF antibody A4.6.1 produced by hybridoma ATCC HB 10709. More preferably the anti-VEGF antibody is a recombinant humanized anti-VEGF monoclonal antibody generated according to Presta et al. (1997) Cancer Res. 57:4593-4599, including but not limited to the antibody known as bevacizumab (BV; Avastin®).

The anti-VEGF antibody "Bevacizumab (BV)", also known as "rhuMAb VEGF" or "Avastin®", is a recombinant humanized anti-VEGF monoclonal antibody generated according to Presta et al. (1997) *Cancer Res.* 57:4593-4599. It comprises mutated human IgG1 framework regions and antigen-binding complementarity-determining regions from the murine anti-hVEGF monoclonal antibody A.4.6.1 that blocks binding of human VEGF to its receptors. Approximately 93% of the amino acid sequence of Bevacizumab, including most of the framework regions, is derived from human IgG1, and about 7% of the sequence is derived from the murine antibody A4.6.1. Bevacizumab has a molecular mass of about 149,000 daltons and is glycosylated.

A "VEGF antagonist" refers to a molecule capable of neutralizing, blocking, inhibiting, abrogating, reducing or interfering with VEGF activities including its binding to one or more VEGF receptors. VEGF antagonists include anti-VEGF antibodies and antigen-binding fragments thereof, receptor molecules and derivatives which bind specifically to VEGF thereby sequestering its binding to one or more receptors, anti-VEGF receptor antibodies and VEGF receptor antagonists such as small molecule inhibitors of the VEGFR tyrosine kinases.

An "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or a co-segregate or manifestation thereof or resulting condition therefrom. Examples of autoimmune diseases or disorders include, but are not limited to arthritis (rheumatoid arthritis, juvenile-onset rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), psoriasis, dermatitis including atopic dermatitis, chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, toxic epidermal necrolysis, scleroderma (including systemic scleroderma), sclerosis such as progressive systemic sclerosis, inflammatory bowel disease (IBD) (for example, Crohn's disease, ulcerative colitis, autoimmune inflammatory bowel disease), pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, episcleritis), respiratory distress syndrome, including adult respiratory distress syndrome (ARDS), meningitis, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis such as Rasmussen's encephalitis, uveitis or autoimmune uveitis, colitis such as microscopic colitis and collagenous colitis, glomerulonephritis (GN) such as membranous GN (membranous nephropathy), idiopathic membranous GN, membranous proliferative GN (MPGN), including Type I and Type II, and rapidly progressive GN, allergic conditions, allergic reaction, eczema, asthma, conditions involving infiltration of T cells and chronic inflammatory responses, atherosclerosis, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) such as cutaneous SLE, subacute cutaneous lupus erythematosus, lupus (including nephritis, cerebritis, pediatric, non-renal, discoid, alopecia), juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), adult onset diabetes mellitus (Type II diabetes), multiple sclerosis (MS) such as spino-optical MS, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitis (including large vessel vasculitis (including polymyalgia rheumatica and giant cell (Takayasu's) arteritis), medium vessel vasculitis (including Kawasaki's disease and polyarteritis nodosa), CNS vasculitis, systemic necrotizing vasculitis, and ANCA-associated vasculitis , such as Churg-Strauss vasculitis or syndrome

(CSS)), temporal arteritis, aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia, pure red cell aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome, antigen-antibody complex mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet's or Behcet's disease, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous, pemphigus (including vulgaris, foliaceus, and pemphigus mucus-membrane pemphigoid), autoimmune polyendocrinopathies, Reiter's disease, immune complex nephritis, chronic neuropathy such as IgM polyneuropathies or IgM-mediated neuropathy, thrombocytopenia (as developed by myocardial infarction patients, for example), including thrombotic thrombocytopenic purpura (TTP) and autoimmune or immune-mediated thrombocytopenia such as idiopathic thrombocytopenic purpura (ITP) including chronic or acute ITP, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune endocrine diseases including thyroiditis such as autoimmune thyroiditis, chronic thyroiditis (Hashimoto's thyroiditis), or subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Addison's disease, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis such as allergic encephalomyelitis, myasthenia gravis, cerebellar degeneration, limbic and/or brainstem encephalitis, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis, bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barré syndrome, Berger's disease (IgA nephropathy), primary biliary cirrhosis, celiac sprue (gluten enteropathy), refractory sprue, dermatitis herpetiformis, cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune inner ear disease (AIED), or autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory polychondritis, pulmonary alveolar proteinosis, amyloidosis, giant cell hepatitis, scleritis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis (e.g., benign monoclonal gammopathy and monoclonal gammopathy of undetermined significance, MGUS), peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular

disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal segmental glomerulosclerosis (FSGS), endocrine ophthalmopathy, uveoretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases, Dressler's syndrome, alopecia arcata, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), male and female autoimmune infertility, ankylosing spondylitis, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, echovirus infection, cardiomyopathy, Alzheimer's disease, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, and giant cell polymyalgia.

A "tumor necrosis factor alpha (TNFα)" refers to a human TNFα molecule comprising the amino acid sequence as described in Pennica *et al., Nature,* 312:721 (1984) or Aggarwal *et al., JBC*, 260:2345 (1985).

A "TNFα inhibitor" herein is an agent that decreases, inhibits, blocks, abrogates or interferes a biological function of TNFα, generally through binding to TNFα and neutralizing its activity. Examples of TNF inhibitors specifically contemplated herein are Etanercept (ENBREL®), Infliximab (REMICADE®) and Adalimumab (HUMIRA™).

The term "inadequate response to a TNFα-inhibitor" refers to an inadequate response to previous or current treatment with a TNFα-inhibitor because of toxicity and/or inadequate efficacy. The inadequate response can be assessed by a clinician skilled in treating the disease in question.

A mammal who experiences "toxicity" from previous or current treatment with the TNFα-inhibitor experiences one or more negative side-effects associated therewith such as infection (especially serious infections), congestive heart failure, demyelination (leading to multiple sclerosis), hypersensitivity, neurologic events, autoimmunity, non-Hodgkin's lymphoma, tuberculosis (TB), autoantibodies, etc.

A mammal who has "failed prior treatment" or experiences "inadequate efficacy" continues to have active disease following previous or current treatment with a drug such as a DMARD or a TNFα-inhibitor. For instance, the patient may have active disease activity after 1 month or 3 months of therapy with the DMARD (such as MTX) or the TNFα-inhibitor.

A "B cell surface marker" herein is an antigen expressed on the surface of a B cell which can be targeted with an antagonist which binds thereto. Exemplary B cell surface markers include the CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD53, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD81, CD82, CD83, CDw84, CD85 and CD86 leukocyte surface markers. The B cell surface marker of particular interest is preferentially expressed on B cells compared to other non-B cell tissues of a mammal and may be expressed on both precursor B cells and mature B cells. In one embodiment, the marker is one, like CD20 or CD19, which is found on B cells throughout differentiation of the lineage from the stem cell stage up to a point just prior to terminal differentiation into plasma cells. The preferred B cell surface markers herein is CD20.

The "CD20" antigen is a ~35 kDa, non-glycosylated phosphoprotein found on the surface of greater than 90% of B cells from peripheral blood or lymphoid organs. CD20 is expressed during early pre-B cell development and remains until plasma cell differentiation. CD20 is present on both normal B cells as well as malignant B cells. Other names for CD20 in the literature include "B-lymphocyte-restricted antigen" and "Bp35". The CD20 antigen is described in Clark *et al. PNAS (USA)* 82:1766 (1985), for example.

"Growth inhibitory" antagonists are those which prevent or reduce proliferation of a cell expressing an antigen to which the antagonist binds. For example, the antagonist may prevent or reduce proliferation of B cells *in vitro* and/or *in vivo*.

The term "antibody" herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

"Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each

light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for

Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

"Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al., Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being

obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al., Nature*, 256:495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al., Nature,* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.*, 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al., Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (*e.g.* Old World Monkey, such as baboon, rhesus or cynomolgus monkey) and human constant region sequences (US Pat No. 5,693,780).

"Humanized" forms of non-human (*e.g.,* murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al., Nature* 321:522-525 (1986); Riechmann *et al., Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.* residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*e.g.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

An antagonist "which binds" an antigen of interest, *e.g.* VEGF, is one capable of binding that antigen with sufficient affinity and/or avidity such that the antagonist is useful as a therapeutic agent for targeting the antigen or a cell expressing the antigen.

An "isolated" antagonist is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antagonist, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antagonist will be purified (1) to greater than 95% by weight of antagonist as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antagonist includes the antagonist *in situ* within recombinant cells since at least one component of the antagonist's natural environment will not be present. Ordinarily, however, isolated antagonist will be prepared by at least one purification step.

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

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disease or disorder as well as those in which the disease or disorder is to be prevented. Hence, the mammal may have been diagnosed as having the disease or disorder or may be predisposed or susceptible to the disease.

The expression "therapeutically effective amount" refers to an amount of the antagonist which is effective for preventing, ameliorating or treating the autoimmune disease in question.

The term "immunosuppressive agent" as used herein for adjunct therapy refers to substances that act to suppress or mask the immune system of the mammal being treated herein. This would include substances that suppress cytokine production, downregulate or suppress self-antigen expression, or mask the MHC antigens. Examples of such agents include 2-amino-6-aryl-5-substituted pyrimidines (see U.S. Pat. No. 4,665,077, the disclosure of which is incorporated herein by reference); nonsteroidal antiinflammatory drugs (NSAIDs); azathioprine; cyclophosphamide; bromocryptine; danazol; dapsone; glutaraldehyde (which masks the MHC antigens, as described in U.S. Pat. No. 4,120,649); anti-idiotypic antibodies for MHC antigens and MHC fragments; cyclosporin A; steroids such as glucocorticosteroids, *e.g.*, prednisone, methylprednisolone, and dexamethasone; methotrexate (oral or subcutaneous); hydroxycloroquine; sulfasalazine; leflunomide; cytokine or cytokine receptor antagonists including anti-interferon-γ, -β, or -α antibodies, anti-tumor necrosis factor-α antibodies (infliximab or adalimumab), anti-TNFα immunoahesin (etanercept), anti-tumor necrosis factor-β antibodies, anti-interleukin-2 antibodies and anti-IL-2 receptor antibodies; anti-LFA-1 antibodies, including anti-CD11a and anti-CD18 antibodies; anti-L3T4 antibodies; heterologous anti-lymphocyte globulin; pan-T antibodies, preferably anti-CD3 or anti-CD4/CD4a antibodies; soluble peptide containing a LFA-3 binding domain (WO 90/08187 published 7/26/90); streptokinase; TGF-β; streptodornase; RNA or DNA from the host; FK506; RS-61443; deoxyspergualin; rapamycin; T-cell receptor (Cohen *et al.*, U.S. Pat. No. 5,114,721); T-cell receptor fragments (Offner *et al.*, *Science*, 251: 430-432 (1991); WO 90/11294; Ianeway, *Nature*, 341: 482 (1989); and WO 91/01133); and T cell receptor antibodies (EP 340,109) such as T10B9.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.* At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen

mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.* paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, NJ) and doxetaxel (TAXOTERE®, Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-15; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, *e.g.*, Wilman, "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions*, 14, pp. 375-382, 615th Meeting Belfast (1986) and Stella *et al.*, "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery*, Borchardt *et al.*, (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as the antagonists disclosed herein and, optionally, a chemotherapeutic agent) to a mammal. The components of the

liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

The term "intravenous infusion" refers to introduction of a drug into the vein of an animal or human patient over a period of time greater than approximately 5 minutes, preferably between approximately 30 to 90 minutes, although, according to the invention, intravenous infusion is alternatively administered for 10 hours or less.

The term "intravenous bolus" or "intravenous push" refers to drug administration into a vein of an animal or human such that the body receives the drug in approximately 15 minutes or less, preferably 5 minutes or less.

The term "subcutaneous administration" refers to introduction of a drug under the skin of an animal or human patient, preferable within a pocket between the skin and underlying tissue, by relatively slow, sustained delivery from a drug receptacle. The pocket may be created by pinching or drawing the skin up and away from underlying tissue.

The term "subcutaneous infusion" refers to introduction of a drug under the skin of an animal or human patient, preferably within a pocket between the skin and underlying tissue, by relatively slow, sustained delivery from a drug receptacle for a period of time including, but not limited to, 30 minutes or less, or 90 minutes or less. Optionally, the infusion may be made by subcutaneous implantation of a drug delivery pump implanted under the skin of the animal or human patient, wherein the pump delivers a predetermined amount of drug for a predetermined period of time, such as 30 minutes, 90 minutes, or a time period spanning the length of the treatment regimen.

The term "subcutaneous bolus" refers to drug administration beneath the skin of an animal or human patient, where bolus drug delivery is preferably less than approximately 15 minutes, more preferably less than 5 minutes, and most preferably less than 60 seconds. Administration is preferably within a pocket between the skin and underlying tissue, where the pocket is created, for example,- by pinching or drawing the skin up and away from underlying tissue.

II. Production of Antagonists

The methods and articles of manufacture of the present invention use, or incorporate, an angiogenesis antagonist. Accordingly, methods for generating such antagonists will be described here.

The angiogenesis antagonist can be a protein antagonist of an angiogenic factor. Preferably the antagonist is a VEGF antagonist. In addition to anti-VEGF antibody, which is a preferred VEGF antagonist for the purpose of this invention, other VEGF antagonists include VEGF variants, soluble VEGF receptor fragments, aptamers capable of blocking VEGF or VEGFR, neutralizing anti-VEGFR antibodies, and low molecule weight inhibitors of VEGFR tyrosine kinases.

A description follows as to exemplary techniques for the production of the antibody antagonists used in accordance with the present invention.

(i) Polyclonal antibodies

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

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al., Nature,* 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

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

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

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al., Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

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

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

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

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

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be

placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al., Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al., Nature*, 348:552-554 (1990). Clackson *et al., Nature*, 352:624-628 (1991) and Marks *et al., J. Mol. Biol.*, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al., Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al., Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

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

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

(iii) Humanized antibodies

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

corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

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

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

(iv) Human antibodies

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al., Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al., Nature*,

362:255-258 (1993); Bruggermann *et al., Year in Immuno.*, 7:33 (1993); and US Patent Nos. 5,591,669, 5,589,369 and 5,545,807.

Alternatively, phage display technology (McCafferty *et al.*, *Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for their review see, *e.g.*, Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). See, also, US Patent Nos. 5,565,332 and 5,573,905.

Human antibodies may also be generated by *in vitro* activated B cells (see US Patents 5,567,610 and 5,229,275).

(v) Antibody fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan *et al., Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.*, Bio/Technology 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; US Patent No. 5,571,894; and US Patent No. 5,587,458. The antibody fragment may also be a "linear antibody", *e.g.*, as described in US Patent 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

(vi) Bispecific antibodies

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

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh *et al., Methods in Enzymology*, 121:210 (1986). According to another approach described in US Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface

comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.* tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al., Science*, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli,* which can be chemically coupled to form bispecific antibodies. Shalaby *et al., J. Exp. Med.*, 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al., J. Immunol.*, 148(5):1547-1553

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

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al. J. Immunol.* 147: 60 (1991).

III. Conjugates and Other Modifications of the Antagonist

The antagonist used in the methods or included in the articles of manufacture herein is optionally conjugated to a cytotoxic agent.

Chemotherapeutic agents useful in the generation of such antagonist-cytotoxic agent conjugates have been described above.

Conjugates of an antagonist and one or more small molecule toxins, such as a calicheamicin, a maytansine (US Patent No. 5,208,020), a trichothene, and CC1065 are also contemplated herein. In one embodiment of the invention, the antagonist is conjugated to one or more maytansine molecules (*e.g.* about 1 to about 10 maytansine molecules per antagonist molecule). Maytansine may, for example, be converted to May-SS-Me which may be reduced to May-SH3 and reacted with modified antagonist (Chari *et al. Cancer Research* 52: 127-131 (1992)) to generate a maytansinoid-antagonist conjugate.

Alternatively, the antagonist is conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin which may be used include, but are not limited to, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I_1 (Hinman *et al. Cancer Research* 53: 3336-3342 (1993) and Lode *et al. Cancer Research* 58: 2925-2928 (1998)).

Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and

PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published October 28, 1993.

The present invention further contemplates antagonist conjugated with a compound with nucleolytic activity (*e.g.* a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

A variety of radioactive isotopes are available for the production of radioconjugated antagonists. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu.

Conjugates of the antagonist and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antagonist. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker or disulfide-containing linker (Chari *et al. Cancer Research* 52: 127-131 (1992)) may be used. Alternatively, a fusion protein comprising the antagonist and cytotoxic agent may be made, *e.g.* by recombinant techniques or peptide synthesis.

The antagonists of the present invention may also be conjugated with a prodrug-activating enzyme which converts a prodrug (*e.g.* a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of such conjugates includes any enzyme capable of acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form. Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free

drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328: 457-458 (1987)).

The enzymes of this invention can be covalently bound to the antagonist by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antagonist of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, *e.g.*, Neuberger *et al.*, *Nature*, 312: 604-608 (1984)).

Other modifications of the antagonist are contemplated herein. For example, the antagonist may be linked to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol.

The antagonists disclosed herein may also be formulated as liposomes. Liposomes containing the antagonist are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al., Proc. Natl Acad. Sci. USA*, 77:4030 (1980); U.S. Pat. Nos. 4,485,045 and 4,544,545; and WO97/38731 published October 23, 1997. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of an antibody of the present invention can be conjugated to the liposomes as described in Martin *et al. J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon *et al. J. National Cancer Inst.*81(19)1484 (1989).

Amino acid sequence modification(s) of protein or peptide antagonists described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antagonist. Amino acid sequence variants of the antagonist are prepared by introducing appropriate nucleotide changes into the antagonist

nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antagonist. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antagonist, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the antagonist that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed antagonist variants are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antagonist with an N-terminal methionyl residue or the antagonist fused to a cytotoxic polypeptide. Other insertional variants of the antagonist molecule include the fusion to the N– or C-terminus of the antagonist of an enzyme, or a polypeptide which increases the serum half-life of the antagonist.

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antagonist molecule replaced by different residue. The sites of greatest interest for substitutional mutagenesis of antibody antagonists include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in the biological properties of the antagonist are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

Any cysteine residue not involved in maintaining the proper conformation of the antagonist also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antagonist to improve its stability (particularly where the antagonist is an antibody fragment such as an Fv fragment).

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody. Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants is affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.* binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or in additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

Another type of amino acid variant of the antagonist alters the original glycosylation pattern of the antagonist. By altering is meant deleting one or more carbohydrate moieties found in the antagonist, and/or adding one or more glycosylation sites that are not present in the antagonist.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antagonist is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antagonist (for O-linked glycosylation sites).

Nucleic acid molecules encoding amino acid sequence variants of the antagonist are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antagonist.

It may be desirable to modify the antagonist of the invention with respect to effector function, *e.g.* so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antagonist. This may be achieved by introducing one or more amino acid substitutions in an Fc region of an antibody antagonist. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al. Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may

thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al. Anti-Cancer Drug Design* 3:219-230 (1989).

To increase the serum half life of the antagonist, one may incorporate a salvage receptor binding epitope into the antagonist (especially an antibody fragment) as described in US Patent 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

IV. Pharmaceutical Formulations

Therapeutic formulations of the antagonists used in accordance with the present invention are prepared for storage by mixing an antagonist having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

Lyophilized formulations adapted for subcutaneous administration are described in WO97/04801. Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the mammal to be treated herein.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a cytotoxic agent, chemotherapeutic agent, cytokine or immunosuppressive agent (*e.g.* one which acts on T cells, such as cyclosporin or an antibody that binds T cells, *e.g.* one which binds LFA-1). The effective amount of such other agents depends on the amount of antagonist present in the formulation, the type of disease or disorder or treatment, and other

factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antagonist, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

V. Treatment with the Antagonist

The present invention concerns therapy of a subpopulation of mammals, especially humans, with, or susceptible to, an autoimmune disease, who has failed or experience an inadequate response to previous or current treatment. Generally, the mammal to be treated herein will be identified following therapy with one or more treatments with one or more DMARDs or one or more TNFα-inhibitor(s), as experiencing an inadequate response to previous or current treatment because of toxicity and/or inadequate efficacy. However, the invention is not limited to a prior therapy step with such a treatment; for instance, the patient may be considered to be prone to experience a toxicity, *e.g.* cardiac toxicity, with a DMARD or a TNFα-inhibitor before therapy therewith has begun, or the patient may be determined to be one who is unlikely to respond to such therapy.

The various autoimmune diseases to be treated herein are listed in the definitions section above. The preferred indications herein are rheumatoid arthritis, lupus, psoriatic arthritis, multiple sclerosis or Crohn's disease.

For the prevention or treatment of disease, the appropriate dosage of antagonist will depend on the type of disease to be treated, as defined above, the severity and course of the

disease, whether the antagonist is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antagonist, and the discretion of the attending physician. The antagonist is suitably administered to the patient at one time or over a series of treatments. In a combination therapy regimen, the compositions of the present invention are administered in a therapeutically effective or synergistic amount. As used herein, a therapeutically effective amount is such that co-administration of the antagonist and one or more other therapeutic agents, or administration of a composition of the present invention, results in reduction or inhibition of the targeting disease or condition. A therapeutically synergistic amount is that amount of antagonist and one or more other therapeutic agents necessary to synergistically or significantly reduce or eliminate conditions or symptoms associated with a particular disease.

Depending on the type and severity of the disease, about 1 μg/kg to 50 mg/kg (*e.g.* 0.1-20 mg/kg) of antagonist is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to about 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. In a preferred aspect, the antagonist is administered every two to three weeks, at a dose ranged from about 1.5 mg/kg to about 15 mg/kg. More preferably, such dosing regimen is used in combination with another therapeutic agent for autoimmune diseases. The progress of the therapy of the invention is easily monitored by conventional techniques and assays.

As noted above, however, these suggested amounts of antagonist are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained, as indicated above. For example, relatively higher doses may be needed initially for the treatment of ongoing and acute diseases. To obtain the most efficacious results, depending on the disease or disorder, the antagonist is administered as close to the first sign, diagnosis, appearance, or occurrence of the disease or disorder as possible or during remissions of the disease or disorder.

The antagonist is administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local immunosuppressive treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the antagonist may suitably be administered by pulse infusion, *e.g.*, with declining doses of the antagonist. Preferably the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

One may administer other compounds, such as cytotoxic agents, chemotherapeutic agents, immunosuppressive agents and/or cytokines with the antagonists herein. The combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. For RA, and other autoimmune diseases, the antagonist (*e.g.* anti-VEGF antibody) may be combined with any one or more of disease-modifying antirheumatic drugs (DMARDs) such as hydroxycloroquine, sulfasalazine, methotrexate, leflunomide, azathioprine, D-penicillamine, Gold (oral), Gold (intramuscular), minocycline, cyclosporine, Staphylococcal protein A immunoadsorption; intravenous immunoglobulin (IVIG); nonsteroidal antiinflammatory drugs (NSAIDs); glucocorticoid (*e.g.* via joint injection); corticosteroid (*e.g.* methylprednisolone and/or prednisone); folate etc. The most preferred DMARD is MTX. Low-dose MTX therapy, administered weekly, inhibits DNA and RNA synthesis, accounting for its antiproliferative effects, and stimulates the release of adenosine, a mediator with anti-inflammatory activity. Adverse effects of MTX include nausea, diarrhea, fatigue, mouth ulcers, and hematologic suppression. Rarely, patients may develop a pneumonia-like reaction or cirrhosis. Methotrexate is usually initiated at a dose of 7.5 to 10 mg per week. The dose is increased as tolerated during the next several months, up to 20 to 25 mg per week. However, lower MTX doses should be prescribed to the elderly and those patients with mild renal dysfunction; MTX should not be given to patients with a serum creatinine level higher than 2.5 mg/dL. The ACR has established guidelines for monitoring patients receiving MTX, recommending that blood cell counts and liver enzymes be assessed at 4- to 8-week intervals.

In another embodiment, the angiogenesis antagonist is used in combination with other antagonist biologics that are effective in treating autoimmune diseases. For example, the angiogenesis antagonist can be used in combination with a TNFα-inhibitor, a B-cell antagonist, or both. A TNFα-inhibitor can be any agent that decreases, inhibits, blocks, abrogates or interferes a biological function of TNFα. Preferably, a TNFα-inhibitor binds to TNFα and neutralizes its activity. Examples of TNF inhibitors specifically contemplated herein are Etanercept (ENBREL®), Infliximab (REMICADE®) and Adalimumab (HUMIRA™). A B-cell antagonist can be an antagonist antibody that binds to a B-cell surface marker such as CD20, CD22, CD19 and CD40. Examples of antibodies which bind the CD20 antigen include: "C2B8" which is now called "rituximab" ("RITUXAN®") (US Patent No. 5,736,137, expressly incorporated herein by reference); the yttrium-[90]-labeled 2B8 murine antibody designated "Y2B8" (US Patent No. 5,736,137, expressly incorporated herein by reference); murine IgG2a "B1" optionally labeled with ^{131}I to generate the "^{131}I-B1"

antibody (BEXXAR™) (US Patent No. 5,595,721, expressly incorporated herein by reference); murine monoclonal antibody "1F5" (Press *et al. Blood* 69(2):584-591 (1987)); "chimeric 2H7 antibody" (US Patent No. 5,677,180, expressly incorporated herein by reference); "humanized 2H7 v16" (see below); huMax-CD20 (Genmab, Denmark); AME-133 (Applied Molecular Evolution); and monoclonal antibodies L27, G28-2, 93-1B3, B-C1 or NU-B2 available from the International Leukocyte Typing Workshop (Valentine *et al.*, In: *Leukocyte Typing* III (McMichael, Ed., p. 440, Oxford University Press (1987)). Examples of antibodies which bind the CD19 antigen include the anti-CD19 antibodies in Hekman *et al. Cancer Immunol. Immunother.* 32:364-372 (1991) and Vlasveld *et al. Cancer Immunol. Immunother.* 40:37-47 (1995); and the B4 antibody in Kiesel *et al. Leukemia Research II*, 12: 1119 (1987).

Aside from administration of protein antagonists to the patient the present application contemplates administration of antagonists by gene therapy. Such administration of nucleic acid encoding the antagonist is encompassed by the expression "administering a therapeutically effective amount of an antagonist". See, for example, WO96/07321 published March 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the patient's cells; *in vivo* and *ex vivo*. For *in vivo* delivery the nucleic acid is injected directly into the patient, usually at the site where the antagonist is required. For *ex vivo* treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells and the modified cells are administered to the patient either directly or, for example, encapsulated within porous membranes which are implanted into the patient (see, *e.g.* U.S. Patent Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. A commonly used vector for *ex vivo* delivery of the gene is a retrovirus.

The currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated virus) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are DOTMA, DOPE and DC-Chol, for example). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.* capsid proteins or

fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, *J. Biol. Chem.* 262:4429-4432 (1987); and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 87:3410-3414 (1990). For review of the currently known gene marking and gene therapy protocols see Anderson *et al.*, *Science* 256:808-813 (1992). See also WO 93/25673 and the references cited therein.

Further details of the invention are illustrated by the following non-limiting Examples. The disclosures of all citations in the specification are expressly incorporated herein by reference.

Example 1

A patient with active rheumatoid arthritis who has failed prior therapy and currently has an inadequate response to MTX is treated with an anti-hVEGF monoclonal antibody such as Avastin®.

Candidates for therapy according to this example include those who were diagnosed with RA for at least six months, according to the revised 1987 ACR criteria. The patients must have received MTX at a dose of 10-25 mg/week per oral or parenteral for at least twelve weeks, with the last four weeks prior to screening at a stable dose. Also, the patients must have failed treatment (lack of efficacy or tolerability) with no more than five DMARDs or biologics (including MTX).

Patients may have swollen joint count (SJC) no less than 6 (66 joint count), and tender joint count (TJC) no less than 6 (68 joint count) at screening and randomization; either CRP no less than 1.2mg/dl (12 mg/L) or ESR no less than 28 mm/h. Patients are preferably between 18 and 64 (inclusive) years old, with less then 5 years since RA diagnosis. Males of reproductive potential preferably use a reliable means of contraception (e.g., physical barrier), and females are preferably post-menopausal or surgically sterilized. Major exclusion criteria are based on concerns of general safety such as evidence of significant uncontrolled concomitant diseases including but not limited to cardiovascular diseases, nervous system, pulmonary, renal, hepatic, endocrine, or gastrointestinal disorders. Also, patients with history of thromboembolic diseases including PE, DVT or CVA, history of diabetes mellitus, history of uncontrolled hypertension or history of proteinuria should be excluded from the treatment.

The anti-VEGF antibody used for therapy is preferably bevacizumab (Avastin® , commercially available from Genentech, Inc.) or a variant thereof having improved binding affinity, inhibitory efficacy or pharmacokinetic properties.

Patients are treated with a therapeutically effective dose of the antibody, for instance, a single dose of 1-2.5 mg/kg i.v. every two weeks (1.0 mg/kg/wk). Patients can also receive

concomitant MTX (10-25 mg/week per oral (p.o.) or parenteral), together with a corticosteroid regimen consisting of methylprednisolone 100 mg i.v. 30 minutes prior to infusions of the anti-VEGF antibody and prednisone 60 mg p.o. on Days 2-7, 30 mg p.o. Days 8-14, returning to baseline dose by Day 16. Patients may also receive folate (5 mg/week) given as either a single dose or as divided daily doses. Patients optionally continue to receive any background corticosteroid (10 mg/d prednisone or equivalent) throughout the treatment period.

The primary endpoint is the proportion of patients with an ACR20 response at Week 24 using a Cochran-Mantel-Haenszel (CMH) test for comparing group differences, adjusted for rheumatoid factor and region.

Additional secondary endpoints include:

1. Proportion of patients with ACR50 and 70 responses at Week 24. These may be analyzed as specified for the primary endpoint.

2. Change in Disease Activity Score (DAS) from screening to Week 24. These may be assessed using an ANOVA model with baseline DAS, rheumatoid factor, and treatment as terms in the model.

3. Categorical DAS responders (EULAR response) at Week 24. These may be assessed using a CMH test adjusted for rheumatoid factor.

4. Changes from screening in ACR core set (SJC, TJC, patient's and physician's global assessments, HAQ, pain, CRP, and ESR). Descriptive statistics may be reported for these parameters.

5. Changes from screening in SF-36. Descriptive statistics are reported for the 8 domain scores and the mental and physical component scores. In addition, the mental and physical component scores are further categorized and analyzed.

6. Change in modified Sharp radiographic total score, erosion score, and joint space narrowing score. These are analyzed using continuous or categorical methodology, as appropriate.

Exploratory endpoints and analysis may involve:

ACR(20/50/70 and ACR n) and change in DAS responses over Weeks 8, 12, 16, 20, 24 and beyond will be assessed using a binary or continuous repeated measures model, as appropriate. Exploratory radiographic analyses including proportion of patients with no erosive progression may be assessed at weeks 24 and beyond.

Further exploratory endpoints (for example complete clinical response, disease free period) will be analyzed descriptively as part of the extended observation period. Changes from Screen in FACIT-F fatigue will be analyzed with descriptive statistics. Therapy of RA with the anti-VEGF antibody in patients with an inadequate response to DMARD or TNFα inhibitor therapy as described above will result in a beneficial clinical response according to any one or more of the endpoints noted above.

What is claimed is:

1. Use of an angiogenesis antagonist in the preparation of a medicament for the treatment of an autoimmune disease in a mammal who has failed prior therapy.

2. The use of claim 1 wherein the angiogenesis antagonist is a VEGF antagonist.

3. The use of claim 1 wherein the antagonist comprises an antibody.

4. The use of claim 3 wherein the antibody is an anti-VEGF antibody.

5. The use of claim 4 wherein the anti-VEGF antibody is bevacizumab.

6. The use of claim 1 wherein the mammal is human.

7. The use of claim 1 wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, juvenile-onset rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis.

8. The use of claim 1 wherein the prior therapy comprises administration of at least one DMARD agent.

9. The use of claim 8 wherein the prior therapy comprises administration of MTX.

10. The use of claim 1 wherein the prior therapy comprises administration of at least one TNFα-inhibitor.

11. The use of claim 1 wherein the angiogenesis antagonist is administered in combination with or in series of a DMARD agent.

12. The use of claim 11 wherein the DMARD agent is MTX.

13. The use of claim 1 wherein the angiogenesis antagonist is administered in combination with or in series of a TNFα-inhibitor.

14. The use of claim 13 wherein the TNFα-inhibitor is selected from the group consisting of etanercept, infliximab and adalimumab.

15. The use of claim 1 wherein the angiogenesis antagonist is administered in combination with or in series of a B-cell antagonist which binds to a B cell surface antigen.

16. The use of claim 15 wherein the B cell surface antigen is selected from the group consisting of CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD53, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD81, CD82, CD83, CDw84, CD85 and CD86.

17. The use of claim 15 wherein the B-cell antagonist comprises an antibody against CD20.

18. The use of claim 17 wherein the antibody against CD20 is rituximab.

19. The use of claim 17 wherein the antibody against CD20 is humanized 2H7 v16.

20. Use of an anti-VEGF antibody in the preparation of a medicament for the treatment of rheumatoid arthritis in a patient who has failed prior DMARD or TNFα-inhibitor therapy and currently has an inadequate response to MTX.

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national application No
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OLIVER S J ET AL: "SUPPRESSION OF COLLAGEN-INDUCED ARTHRITIS USING AN ANGIOGENESIS INHIBITOR, AGM-1470, AND A MICROTUBULE STABILIZER, TAXOL" CELLULAR IMMUNOLOGY, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 157, no. 1, 1 August 1994 (1994-08-01), pages 291-299, XP000612505 ISSN: 0008-8749 abstract page 292 - page 293 ----- -/--	1-20

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

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WILSON JENNIFER FISHER: "Angiogenesis therapy moves beyond cancer." ANNALS OF INTERNAL MEDICINE. 20 JUL 2004, vol. 141, no. 2, 20 July 2004 (2004-07-20), pages 165-168, XP002378544 ISSN: 1539-3704 page 165, right-hand column page 166, right-hand column	1-20
X	MANLEY P W ET AL: "Therapies directed at vascular endothelial growth factor" EXPERT OPINION ON INVESTIGATIONAL DRUGS, ASHLEY PUBLICATIONS LTD., LONDON, GB, vol. 11, no. 12, December 2002 (2002-12), pages 1715-1736, XP002256125 ISSN: 1354-3784 paragraph [03.2] paragraph [4.1.1]	1-20
X	US 2004/120950 A1 (ALITALO KARI ET AL) 24 June 2004 (2004-06-24) claims 1-43	1-20

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

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US 2004120950 A1	24-06-2004	NONE	

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(54) Title: ANTIBODIES AGAINST VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-1

(57) Abstract: Monoclonal antibodies that are specific for vascular endothelial growth factor receptor 1 (VEGFR-1). This invention also provides nucleotide sequences encoding and amino acid sequences comprising variable heavy and light chain immunoglobulin molecules, including sequences corresponding to the complementarity determining regions of CDR1, CDR2, and CDR3. The invention also provides methods for generation and expression of anti-VEGFR-1 antibodies and methods of treating angiogenic-related disorders and reducing tumor growth by administering anti-VEGFR-1 antibodies.

ANTIBODIES AGAINST VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-1

FIELD OF INVENTION

The present invention relates to antibodies that are specific for vascular endothelial growth factor receptor-1 (VEGFR-1) and methods of treating angiogenesis-associated diseases and tumors with antibodies to VEGFR-1.

BACKGROUND OF THE INVENTION

Angiogenesis, which refers to the formation of capillaries from pre-existing vessels in the embryo and adult organism, is known to be a key element in tumor growth, survival and metastasis. Growth factors and their receptors, including epidermal growth factor (EGF), transforming growth factor-α (TGF-α), transforming growth factor-β (TGF-β), acidic and basic fibroblast growth factor (aFGF and bFGF), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), are thought to play a role in tumor angiogenesis. *See* Klagsbrun & D'Amore, Annual Rev. Physiol., 53: 217-239 (1991). Binding of these growth factors to their cell surface receptors induces receptor activation, which initiates and modifies signal transduction pathways and leads to cell proliferation and differentiation. VEGF, an endothelial cell-specific mitogen, is distinct among these factors in that it acts as an angiogenesis inducer by specifically promoting the proliferation of endothelial cells.

The biological response of VEGF is mediated through its high affinity receptors, which are selectively expressed on endothelial cells during embryogenesis (Millauer, *Cell*, 72: 835-846 (1993)) and during tumor formation. VEGF receptors (VEGFRs) typically are class III receptor-type tyrosine kinases characterized by having several, typically 5 or 7, immunoglobulin-like loops in their amino-terminal extracellular receptor ligand-binding domains (Kaipainen *et al.*, *J. Exp. Med.*, 178:2077-2088 (1993)). The other two regions include a transmembrane region and a carboxy-terminal intracellular catalytic domain interrupted by an insertion of hydrophilic interkinase sequences of variable lengths, called the kinase insert domain (Terman *et al.*, *Oncogene*, 6:1677-1683 (1991)). VEGFRs include *fms*-like tyrosine kinase receptor (flt-1), or VEGFR-1, sequenced by Shibuya *et al.*, *Oncogene*, 5: 519-524 (1990), kinase insert domain-containing receptor/fetal liver kinase (KDR/flk-1),

or VEGFR-2, described in WO 92/14248, filed February 20, 1992, and Terman *et al.*, *Oncogene*, 6: 1677-1683 (1991) and sequenced by Matthews *et al.*, *Proc. Natl. Acad. Sci. USA*, 88: 9026-9030 (1991), although other receptors, such as neuropilin-1 and -2, can also bind VEGF. Another tyrosine kinase receptor, VEGFR-3 (flt-4), binds the VEGF homologues VEGF-C and VEGF-D and is more important in the development of lymphatic vessels.

The importance of VEGFR-1 in regulation of pathological angiogenesis has been shown in *in vivo* experimental models. Deficiency of VEGFR-1 tyrosine kinase domain results in decreased blood vessel formation in tumors, indicating a significant role of VEGFR-1 tyrosine kinase in pathological angiogenesis (Hiratsuka *et al.*, *Cancer Research*, 61:1207-1213 (2001)). VEGFR-1 tyrosine kinase domain is also required for promotion of tumor pathogenesis and metastasis by induction of matrix metalloprotease-9 (MMP-9) in endothelial cells and macrophages (Hiratsuka *et al.*, *Cancer Cell*, 2:289-300 (2002)). In addition, VEGFR-1 has been shown to mediate mobilization and differentiation of PlGF responsive BM-derived precursors (Hattori *et al.*, *Nature Medicine*, 8:841-849 (2002)). Inhibition of VEGFR-1 by an anti-VEGFR-1 antibody led to reduction of tumor angiogenesis by preventing recruitment of bone marrow-derived endothelial and monocyte progenitor cells from vascularization in tumors (Lyden *et al.*, *Nature Medicine*, 7:1194-1201 (2001)). Treatment with an anti-VEGFR-1 antibody also effectively inhibited pathological angiogenesis in tumors and ischemic retina in animal models (Lutten *et al.*, *Nature Medicine*, 8:831-840 (2002)).

In addition to the role of VEGFR-1 in angiogenesis, co-expression of VEGF and its receptors is also frequently found in hematological malignant cells and certain solid tumor cells (Bellamy, *Cancer Research*, 59:728-733 (1999); Ferrer *et al.*, *Urology*, 54:567-572 (1999); Price *et al.*, *Cell Growth Differ.*, 12:129-135 (2001)). VEGF has been shown to directly induce proliferation, survival, and invasion of VEGF receptor expressing leukemia cells by activation of downstream intracellular signaling pathways through a ligand stimulated autocrine loop (Dias *et al.*, *Proc Natl Acad Sci USA*, 98:10857-10862 (2001); Gerber *et al.*, *J Mol Med.*, 81:20-31 (2003)). VEGF stimulation also results in an increased invasiveness of the VEGFR-1 expressing breast cancer cells by inducing the activation of ERK1/2 and PI 3/Akt-kinase signaling pathways (Price *et al.*, *Cell Growth Differ.*, 12:129-135 (2001)).

VEGFR-1 and its ligands have also been shown to play and important role in inflammatory disorders. VEGF-B deficiency resulted in the reduction of inflammation-associated vessel density and synovial inflammation in models of arthritis (Mould *et al.*, *Arthritis Rheum.*, 48:2660-2669 (2003)). PlGF also plays a critical tole in the control of cutaneous inflammation by mediating vascular enlargement, inflammatory cells and monocytes/macrophages, and has been shown to contribute to modulation of atherosclerosis and rheumatoid arthritis in animal models (Luttun *et al.*, *Nature Medicine,* 8:831-840 (2002); *Autiero & Thromb Haemost.*, 1:1356-1370 (2003)). Treatment with a neutralizing anti-VEGFR-1 antibody suppressed inflammatory joint destruction in arthritis, reduced atherosclerotic plaque growth and vulnerability. The anti-inflammatory effects of the anti-VEGFR-1 antibody were attributable to a reduced mobilization of bone marrow-derived myeloid progenitors into the peripheral blood, a defective activation of myeloid cells, and an impaired differentiation and infiltration of VEGFR-1-expressing leukocytes in inflamed tissues. Thus, VEGFR-1 may also be therapeutic target for treatment of inflammation-related disorders.

There remains a need for agents which inhibit VEGF receptor activity, such as fully human monoclonal antibodies (mAbs) specific for VEGFR-1. The anti-VEGFR-1 antibodies may be a useful, novel therapeutic antagonist for treatment of angiogenesis-associated diseases and cancer.

BRIEF SUMMARY OF THE INVENTION

In an embodiment, the present invention provides a monoclonal antibody or fragment thereof that specifically bind to VEGFR-1 and comprises a light chain complementarity determining region-2 (CDR2) of SEQ ID NO: 2 and a light chain complementarity region-3 (CDR3) of SEQ ID NO: 3.

In another embodiment, the present invention provides a monoclonal antibody or fragment thereof that specifically binds to VEGFR-1 and is at least 70% homologous to the amino acid sequence of an antibody or fragment thereof that comprises a light chain complementarity determining region-2 (CDR2) of SEQ ID NO: 2 and a light chain complementarity region-3 (CDR3) of SEQ ID NO: 3.

In another embodiment, the present invention provides an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting

of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27. The nucleotide sequence encodes an antibody or fragment thereof that specifically binds to VEGFR-1.

In another embodiment, the present invention provides an isolated polynucleotide comprising a nucleotide sequence that encodes an antibody or fragment thereof that specifically binds to VEGFR-1 and that is at least 70% homologous to the nucleotide sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27.

In another embodiment, the present invention provides a method of inhibiting angiogenesis or reducing tumor growth by administering a therapeutically effective amount of an antibody or fragment thereof that specifically bind to VEGFR-1 and comprises a light chain complementarity determining region-2 (CDR2) of SEQ ID NO: 2 and a light chain complementarity region-3 (CDR3) of SEQ ID NO: 3.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is the amino acid sequences of the light chain variable region and the heavy chain variable region of embodiments of anti-VEGFR-1 antibodies of the present invention.

Figure 2 is the nucleotide sequences of the light chain variable region and the heavy chain variable region of embodiments of anti-VEGFR-1 antibodies of the present invention.

Figure 3 is a chart depicting the results of an ELISA-based binding assay measuring *in vitro* binding activity of embodiments of anti-VEGFR-1 antibodies of the present invention to VEGFR-1.

Figure 4 is a chart depicting the results of an ELISA-based blocking assay measuring *in vitro* competition of embodiments of anti-VEGFR-1 antibodies of the present invention with PlGF for VEGFR-1 binding.

Figure 5 is a chart depicting the results of an ELISA-based blocking assay measuring *in vitro* competition of embodiments of anti-VEGFR-1 antibodies of the present invention with VEGF for VEGFR-1 binding.

Figure 6A-D are charts depicting the results of specificity of anti-VEGFR-1 antibody 18F1 of the present invention with binding of human VEGFR-1 (**Figure**

6A), but not mouse VEGFR-1 (**Figure 6B**), human VEGFR-2 (**Figure 6C**), or mouse VEGFR-2 (**Figure 6D**).

Figure 7A-E are results of flow cytometry analysis showing binding reactivity of embodiments of anti-VEGFR-1 antibodies of the present invention with VEGFR-1 expressing porcine aorta endothelial cells.

Figure 8A-B is results of flow cytometry analysis showing binding reactivity of anti-VEGFR-1 antibody 18F1 of the present invention with VEGFR-1 expressing porcine endothelial cells (**Figure 8A**) and DU4475 human breast carcinoma cells (**Figure 8B**).

Figure 9 is a chart depicting results of a cell-based blocking assay measuring *in vitro* competition of anti-VEGFR-1 antibody 18F1 of the present invention with VEGF binding to VEGFR-1 on endothelial cells.

Figure 10 is a Western blot analysis demonstrating the reduction of PlGF-stimulated phosphorylation of VEGFR-1 by treatment with anti-VEGFR-1 antibody 18F1 of the present invention in porcine aorta endothelial VEGFR-1 expressing cells.

Figure 11 is a Western blot analysis demonstrating inhibition of PlGF or VEGF-stimulated phosphorylation of VEGFR-1 by treatment with anti-VEGFR-1 antibody 18F1 of the present invention in BT474 breast cancer cells.

Figure 12 is a Western blot analysis demonstrating inhibition of PlGF induced activation of ERK1/2 downstream signaling by embodiments of anti-VEGFR-1 antibodies of the present invention in porcine aorta endothelial VEGFR-1 expressing cells.

Figure 13 is a Western blot analysis demonstrating the inhibition of VEGF induced activation of ERK1/2 downstream signaling by embodiments of anti-VEGFR-1 antibodies of the present invention in porcine aorta endothelial VEGFR-1 expressing cells.

Figures 14A-B is a Western blot analysis demonstrating the inhibition of PIGF(**Figure 14A**) or VEGF (**Figure 14B**)-induced activation of ERK 1/2 downstream signaling by anti-VEGFR-1 antibody 18F1 of the present invention in VEGFR-1 expressing porcine aorta endothelial cells.

Figure 15 is a Western blot analysis demonstrating that the anti-VEGFR-1 antibody 18F1 of the present invention blocked PlGF or VEGF-stimulated phosphorylation of Akt in BT474 breast cancer cells.

Figure 16 is a dose response curve showing the inhibition of VEGF stimulated cell proliferation in DU4475 breast carcinoma cells treated with embodiments of anti-VEGFR-1 antibodies of the present invention in a dose response manner.

Figure 17 is a dose response curve showing the inhibition of PlGF stimulated cell proliferation in DU4475 breast carcinoma cells treated with embodiments of anti-VEGFR-1 antibodies of the present invention in a dose response manner.

Figure 18 A-B is a dose response curve showing the inhibition of PIGF (**Figure 18A**) or VEGF (**Figure 18B**)-stimulated cell proliferation in DU4475 breast carcinoma cells treated with anti-VEGFR-1 antibody 18F1 of the present invention in a dose response manner.

Figure 19 A and **19B** are charts plotting tumor growth of DU4475 breast tumors versus days after treatment with embodiments of anti-VEGFR-1 antibodies of the present invention.

Figure 20 A-C is a chart plotting tumor growth of DU4475 (**Figure 20A**), MDA-MB-231 (**Figure 20B**) and MDA-MB-435 (**Figure 20C**) breast tumors versus days after treatment with anti-VEGFR-1 antibody 18F1 of the present invention.

Figure 21A-B is a chart plotting tumor growth of DU4475 (**Figure 21A**) and MDA-MB-231 (**Figure 21B**) breast tumors versus days after treatment with anti-human VEGFR-1 antibody 18F1 of the present invention and anti-mouse VEGFR-1 antibody MF1.

Figure 22 is a chart of the number of colon cancer cell colonies present after treatment with anti-human VEGFR-1 antibody 18F1 in the presence of VEGF-A and VEGF-B.

Figure 23A is a chart of the number of migrated tumor cells after treatment with anti-human VEGFR-1 antibody 18F1 in the presence of VEGF-A and VEGF-B.

Figure 23B are photomicrographs of stained migrated cells after treatment with anti-human VEGFR-1 antibody 18F1 in the presence of VEGF-A and VEGF-B.

Figure 24A is a chart of the number of tumor cells that migrated across a layer of MATRIGEL™ after treatment with anti-human VEGFR-1 antibody 18F1 in the presence of VEGF-A or VEGF-B.

Figure 24B are photomicrographs of stained migrated cells after treatment with anti-human VEGFR-1 antibody 18F1 in the presence of VEGF-A and VEGF-B.

Figure 25 is a chart plotting tumor growth of DU4475 (**Figure 25A**) and MDA-MB-435 (**Figure 25B**) breast tumors versus days after treatment with anti-VEGFR-1 antibodies 18F1, 6F9 and 15F11.

Figure 26 is a chart plotting growth of HT-29 (**Figure 26A**), DLD-1 (**Figure 26B**) and GEO (**Figure 26C**) colon cancer cells versus days after treatment with particular doses of anti-human VEGFR-1 antibody 18F1.

Figure 27 are photomicrographs of MDS-MB-231 xenograft tumors after treatment with anti-human VEGFR-1 antibody 18F1.

Figure 28 is a chart plotting tumor growth versus days after treatment with particular doses of anti-human anti-VEGFR-1 antibody 18F1, anti-mouse anti-VEGFR-1 antibody MF1, or both in MDA-MB-231 (**Figure 28A**) and DU4475 (**Figure 28B**) xenografts.

Figure 29 is a chart plotting tumor growth versus days after treatment with anti-human anti-VEGFR-1 antibody 18F1 and anti-mouse anti-VEGFR-1 antibody MF1 in combination with cyclophosphamide in MDS-MB-231 xenografts.

Figures 30A and **B** are charts plotting tumor growth versus days after treatment with 5-FU/LV or doxorubicin in combination with anti-human anti-VEGFR-1 antibody 18F1 and anti-mouse anti-VEGFR-1 antibody MF1 in MDA-MB-231 xenografts.

Figure 31 is a chart of total tumor cell count versus antibody concentration of various amounts of 18F1 in the presence of VEGF-A (**Figure 31A**) or PIGF (**figure 31B**) following treatment with desferrioxamine.

Figure 32A, **B**, and **C** are charts depicting the specificity of anti-human anti-VEGFR-1 antibody 18F1 and anti-mouse anti-VEGFR-1 antibody MF1.

DETAILED DESCRIPTION OF THE INVENTION

In an embodiment, the present invention provides a monoclonal antibodies and fragments thereof that specifically bind to VEGFR-1 (such antibodies and fragments thereof referred to herein as "anti-VEGFR-1 antibodies" unless otherwise indicated). Anti-VEGFR-1 antibodies of the present invention comprise a light chain complementarity determining region-2 (CDR2) of SEQ ID NO: 2 and a light chain complementarity region-3 (CDR3) of SEQ ID NO: 3. Alternatively and preferably, anti-VEGFR-1 antibodies of the present invention comprise a light chain

complementarity region-1 (CDR1) having the following sequence: RASQSX$_1$SSSYLA, where X$_1$ is V or G (SEQ ID NO: 1 or 4). Alternatively and preferably, anti-VEGFR-1 antibodies of the present invention comprise a heavy chain CDR1 having the following sequence: GFX$_2$FSSYGMH, where X$_2$ is T or A (SEQ ID NO: 5 or 11). Alternatively and preferably, anti-VEGFR-1 antibodies of the present invention comprise a heavy chain CDR2 having the following sequence: VIWX$_3$DGSNKYYADSVX$_4$G, where X$_3$ is Y or F and X$_4$ is K or R (SEQ ID NO: 6, 9, or 12). Alternatively and also preferably, anti-VEGFR-1 antibodies of the present invention comprise a heavy chain CDR3 having the following sequence: DHX$_5$GSGX$_6$HX$_7$YX$_8$YYGX$_9$DV, where X$_5$ is F or Y; X$_6$ is A or V; X$_7$ is Y, S, or H; X$_8$ is Y or F; and X$_9$ is M or L (SEQ ID NO: 7, 8, 10, 13). The amino acid sequences of the CDRs of preferred anti-VEGFR-1 antibodies (designated as clones "6F9," "13G12," "15F11," and "18F1" (or "IMC-18F1")) are set forth below in Table 1.

Table 1 - CDR sequence of anti-VEGFR-1 antibodies

Clone	CDR1	CDR2	CDR3
Light Chain			
6F9	RASQSGSSSYLA (SEQ ID NO:1)	GASSRAT (SEQ ID NO:2)	QQYGSSPLT (SEQ ID NO:3)
13G12	RASQSGSSSYLA (SEQ ID NO:1)	GASSRAT (SEQ ID NO:2)	QQYGSSPLT (SEQ ID NO:3)
15F11	RASQSVSSSYLA (SEQ ID NO:4)	GASSRAT (SEQ ID NO:2)	QQYGSSPLT (SEQ ID NO:3)
18F1	RASQSVSSSYLA (SEQ ID NO:4)	GASSRAT (SEQ ID NO:2)	QQYGSSPLT (SEQ ID NO:3)
Heavy Chain			
6F9	GFTFSSYGMH (SEQ ID NO:5)	VIWYDGSNKYYADSVKG (SEQ ID NO:6)	DHFGSGAHYYYYYGMDV (SEQ ID NO:7)
13G12	GFTFSSYGMH (SEQ ID NO:5)	VIWYDGSNKYYADSVKG (SEQ ID NO:6)	DHYGSGAHYYYYYGMDV (SEQ ID NO:8)
15F11	GFTFSSYGMH (SEQ ID NO:5)	VIWFDGSNKYYADSVKG (SEQ ID NO:9)	DHYGSGAHSYYYYGLDV (SEQ ID NO:10)

18F1	GFAFSSYGMH (SEQ ID NO:11)	VIWYDGSNKYYADSVRG (SEQ ID NO:12)	DHYGSGVHHYFYYGLDV (SEQ ID NO:13)

In another embodiment, anti-VEGFR-1 antibodies of the present invention have a light chain variable region (V_L) of SEQ ID NO:14, 15, or 16 and/or a heavy chain variable region (V_H) of SEQ ID NO:17, 18, 19, or 20. The amino acid sequences of the light and heavy chain variable regions of preferred anti-VEGFR-1 antibodies of the present invention are set forth below in Table 2.

Table 2 – Variable region sequence of anti-VEGFR-1 antibodies (underlined portions represent CDRs)

Clone	Light Chain
6F9	EIVLTQSPGTLSLSPGERATLSCRASQSGSSSYLAWYQQKPGQAPRLLIYGASS RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIK RTVAAPSVFIFP SEQ ID NO: 14
13G12	EIVLTQSPGTLSLSPGERATLSCRASQSGSSSYLAWYQQKPGQAPRLLIYGASS RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIK RTVAAPSVFIFP SEQ ID NO: 14
15F11	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASS RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGQGTRLEIKR TVAAPSVFIFP SEQ ID NO: 15
18F1	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASS RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIK RTVAAPSVFIFP SEQ ID NO: 16
Clone	Heavy Chain
6F9	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVI WYDGSNKYYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYHCTRDHFG SGAHYYYYYGMDVWGQGTTVTVSS SEQ ID NO:17

13G12	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVI WYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDHY GSGAHYYYYYGMDVWGQGTTVTVSS SEQ ID NO:18
15F11	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVI WFDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDHYG SGAHSYYYYGLDVWGQGTSVTVSS SEQ ID NO:19
18F1	QAQVVESGGGVVQSGRSLRLSCAASGFAFSSYGMHWVRQAPGKGLEWVAVI WYDGSNKYYADSVRGRFTISRDNSENTLYLQMNSLRAEDTAVYYCARDHYG SGVHHYFYYGLDVWGQGTTVTVSS SEQ ID NO:20

In a preferred embodiment, the anti-VEGFR-1 antibodies of the present invention are human antibodies.

Anti-VEGFR-1 antibodies of the present invention include whole antibodies and antibody fragments that specifically bind to VEGFR-1. Non-limiting examples of types of antibodies according to the present invention include naturally occurring antibodies; single chain antibodies; multivalent single chain antibodies such as diabodies and tribodies; monovalent fragments such as Fab (Fragment, antigen binding), bivalent fragments such as $(Fab')_2$; Fv (fragment variable) fragments or derivatives thereof such as single chain Fv (scFv) fragments; and single domain antibodies that bind specifically to VEGFR-1.

Naturally occurring antibodies typically have two identical heavy chains and two identical light chains, with each light chain covalently linked to a heavy chain by an interchain disulfide bond and multiple disulfide bonds further linking the two heavy chains to one another. Individual chains can fold into domains having similar sizes (110-125 amino acids) and structures, but different functions. The light chain can comprise one V_L and one constant domain (C_L). The heavy chain can also comprise one V_H and/or depending on the class or isotope of antibody, three or four constant domains (C_H1, C_H2, C_H3, and C_H4). In humans, the isotypes are IgA, IgD, IgE, IgG, and IgM, with IgA and IgG further subdivided into subclasses or subtypes (IgA_{1-2} and IgG_{1-4}).

Single chain antibodies lack some or all of the constant domains of the whole antibodt from which they are derived. The peptide linkers used to produce the single

chain antibodies may be flexible peptides selected to assure that the proper three-dimensional folding of the V_L and V_H domains occurs. Generally, the carboxyl terminus of the V_L or V_H sequence may be covalently linked by such a peptide linker to the amino acid terminus of a complementary VH or VL sequence. The linker is generally 10 to 50 amino acid residues, preferably 10 to 30 amino acid residues, more preferably 12 to 30 amino acid residues, and most preferably 15 to 25 amino acid residues. An example of such linker peptides include $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_3$ (SEQ ID NO: 28).

Multiple single chain antibodies, each single chain having one V_H and one V_L domain covalently linked by a first peptide linker, can be covalently linked by at least one or more peptide linkers to form a multivalent single chain antibody, which can be monospecific or multispecific. Each chain of a mulivalent single chain antibody includes a variable light chain fragment and a variable heavy chain fragment, and is linked by a peptide linker to at least one other chain.

Two single chain antibodies can be combined to form a diabody, also known as a trivalent dimer. Diabodies have two chains and two binding sites and can be monospecific or bispecific. Each chain of the diabody includes a V_H domain connected to a V_L domain. The domains are connected with linkers that are short enough to prevent pairing between domains on the same chain, thus driving the pairing between complementary domains on different chains to recreate the two antigen-binding sites.

Three single chain antibodies can be combined to form triabodies, also known as trivalent trimers. Triabodies are constructed with the amino acid terminus of a V_L or V_H domain directly fused to the carboxyl terminus of a V_L or V_H domain, i.e., without any linker sequence. The triabody has three Fv heads with the polypeptides arranged in a cyclic, head-to-tail fashion. A possible conformation of the triabody is planar with the three binding sites located in a plane at an angle of 120 degrees from one another. Triabodies can be monospecific, bispecific or trispecific.

Fab fragments refer to fragments of the antibody consisting of $V_LC_LV_HC_{H1}$ domains. Those generated by papain digestion are referred to as "Fab" and do not retain the heavy chain hinge region. Those generated by pepsin digestion are referred to either as "$(Fab')_2$," in which case the interchain disulfide bonds are intact, or as

Fab', in which case the disulfide bonds are not retained. Bivalent $(Fab')_2$ fragments have higher avidity for antigen than that of monovalent Fab fragments.

Fv fragments are the portion of an antibody consisting of the V_L and V_H domains and constitute the antigen-binding site. scFv is an antibody fragment containing a V_L domain and V_H domain on one polypeptide chain, wherein the N terminus of one domain and the C terminus of the other domain are joined by a flexible linker to allows the two fragments to associate to form a functional antigen binding site (*see*, for example U.S. Pat. No. 4,946,778 (Ladner *et al.*), WO 88/09344, (Huston *et al.*), both of which are incorporated by reference herein). WO 92/01047 (McCafferty et al.), which is incorporated by reference herein, describes the display of scFv fragments on the surface of soluble recombinant genetic display packages, such as bacteriophage.

Single domain antibodies have a single variable domain that is capable of efficiently binding antigen. Examples of antibodies wherein binding affinity and specificity are contributed primarily by one or the other variable domain are known in the art. *See, e.g.*, Jeffrey, P.D. et al., *Proc. Nat.l. Acad. Sci.* U S A 90:10310-4 (1993), which is incorporated by reference herein and which discloses an anti-digoxin antibody which binds to digoxin primarily by the antibody heavy chain. Accordingly, single antibody domains can be identified that bind well to VEGF receptors. It is understood that, to make a single domain antibody from an antibody comprising a V_H and a V_L domain, certain amino acid substitutions outside the CDR regions may be desired to enhance binding, expression or solubility. For example, it may be desirable to modify amino acid residues that would otherwise be buried in the V_H-V_L interface.

Each domain of anti-VEGFR-1 antibodies of the present invention may be a complete antibody heavy or light chain variable domain, or it may be a functional equivalent or a mutant or derivative of a naturally occuring domain, or a synthetic domain constructed, for example, *in vitro* using a technique such as one described in WO 93/11236 (Griffiths et al.). For instance, it is possible to join together domains corresponding to antibody variable domains which are missing at least one amino acid. The important characterizing feature is the ability of each domain to associate with a complementary domain to form an antigen binding site. Accordingly, the terms "variable heavy/light chain fragment" should not be construed to exclude variants which do not have a material effect on VEGFR-1 binding specificity.

As used herein, an "anti-VEGFR-1 antibody" include modifications of an anti-VEGFR-1 antibody of the present invention that retain specificity for VEGFR-1. Such modifications include, but are not limited to, conjugation to an effector molecule such as a chemotherapeutic agent (e.g., cisplatin, taxol, doxorubicin) or cytotoxin (e.g., a protein, or a non-protein organic chemotherapeutic agent). Modifications further include, but are not limited to conjugation to detectable reporter moieties. Modifications that extend antibody half-life (e.g., pegylation) are also included.

Proteins and non-protein agents may be conjugated to the antibodies by methods that are known in the art. Conjugation methods include direct linkage, linkage via covalently attached linkers, and specific binding pair members (e.g., avidin-biotin). Such methods include, for example, that described by Greenfield et al., *Cancer Research* 50, 6600-6607 (1990), which is incorporated by reference herein, for the conjugation of doxorubicin and those described by Arnon et al., *Adv. Exp. Med. Biol.* 303, 79-90 (1991) and by Kiseleva *et al., Mol. Biol.* (USSR)25, 508-514 (1991), both of which are incorporated by reference herein, for the conjugation of platinum compounds.

Anti-VEGFR-1 antibodies of the present invention also include those for which binding characteristics have been improved by direct mutation, methods of affinity maturation, phage display, or chain shuffling. Affinity and specificity may be modified or improved by mutating any of the CDRs of the antibodies of the present invention and screening for antigen binding sites having the desired characteristics (*see, e.g.*, Yang *et al.*, *J. Mol. Biol.*, 254: 392-403 (1995), which is incorporated by reference herein). The CDRs may be mutated in a variety of ways that are known to one of skill in the art. For example, one way is to randomize individual residues or combinations of residues so that in a population of otherwise identical antigen binding sites, all twenty amino acids are found at particular positions. Alternatively, mutations are induced over a range of CDR residues by error prone PCR methods (*see, e.g.*, Hawkins et al., *J. Mol. Biol.*, 226: 889-896 (1992), which is incorporated by reference herein). For example, phage display vectors containing heavy and light chain variable region genes may be propagated in mutator strains of *E. coli* (*see, e.g.*, Low et al., *J. Mol. Biol.*, 250: 359-368 (1996), which is incorporated by reference herein).

Anti-VEGFR-1 antibodies also include functional equivalents that include polypeptides with amino acid sequences substantially the same as the amino acid sequence of the variable or hypervariable regions of the antibodies of the present invention. "Substantially the same" amino acid sequence includes an amino acid sequence with at least 70%, preferably at least 80%, and more preferably at least 90% identity to another amino acid sequence when the amino acids of the two sequences are optimally aligned and compared to determine exact matches of amino acids between the two sequences. "Substantially the same" amino acid sequence also includes an amino acid sequence with at least 70%, preferably at least 80%, and more preferably at least 90% homology to another amino acid sequence, as determined by the FASTA search method in accordance with Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85, 2444-8 (1988).

As stated earlier, anti-VEGFR-1 antibodies of the present invention specifically bind to VEGFR-1. Such antibodies can be monospecific or bispecific so long as one antigen-binding site is specific for VEGFR-1. Antibody specificity, which refers to selective recognition of an antibody for a particular epitope of an antigen, of antibodies for VEGFR-1 can be determined based on affinity and/or avidity. Affinity, represented by the equilibrium constant for the dissociation of an antigen with an antibody (K_d), measures the binding strength between an antigenic determinant (epitope) and an antibody binding site. Avidity is the measure of the strength of binding between an antibody with its antigen. Antibodies typically bind with a K_d of 10^{-5} to 10^{-11} liters/mole. Any K_d less than 10^{-4} liters/mole is generally considered to indicate non-specific binding. The lesser the value of the K_d, the stronger the binding strength between an antigenic determinant and the antibody binding site.

Anti-VEGFR-1 antibodies of the present invention specifically bind to the extracellular region of VEGFR-1 and preferably neutralize activation of VEGFR-1 by preventing binding of a ligand of VEGFR-1 to the receptor. In such preferable embodiments, the antibody binds VEGFR-1 at least as strongly as the natural ligands of VEGFR-1 (including VEGF(A), VEGF-B and PIGF).

Neutralizing activation of VEGFR-1 includes diminishing, inhibiting, inactivating, and/or disrupting one or more of the activities associated with signal transduction. Such activities include receptor dimerization, autophosphorylation of

VEGFR-1, activation of VEGFR-1's internal cytoplasmic tyrosine kinase domain, and initiation of multiple signal transduction and transactivation pathways involved in regulation of DNA synthesis (gene activation) and cell cycle progression or division. One measure of VEGFR-1 neutralization is inhibition of the tyrosine kinase activity VEGFR-1. Tyrosine kinase inhibition can be determined using well-known methods such as phosphorylation assays which measuring the autophosphorylation level of recombinant kinase receptor, and/or phosphorylation of natural or synthetic substrates. Phosphorylation can be detected, for example, using an antibody specific for phosphotyrosine in an ELISA assay or on a western blot. Some assays for tyrosine kinase activity are described in Panek *et al.*, *J. Pharmacol. Exp. Thera.*, 283: 1433-44 (1997) and Batley *et al.*, *Life Sci.*, 62: 143-50 (1998), both of which are incorporated by reference.

In addition, methods for detection of protein expression can be utilized to determine whether an antibody neutralizes activation of VEGFR-1, wherein the proteins being measured are regulated by VEGFR-1 tyrosine kinase activity. These methods include immunohistochemistry (IHC) for detection of protein expression, fluorescence *in situ* hybridization (FISH) for detection of gene amplification, competitive radioligand binding assays, solid matrix blotting techniques, such as Northern and Southern blots, reverse transcriptase polymerase chain reaction (RT-PCR) and ELISA. *See, e.g.*, Grandis et al., *Cancer*, 78:1284-92. (1996); Shimizu et al., *Japan J. Cancer Res.*, 85:567-71 (1994); Sauter et al., *Am. J. Path.*, 148:1047-53 (1996); Collins, Glia, 15:289-96 (1995); Radinsky et al., *Clin. Cancer Res.*, 1:19-31 (1995); Petrides et al., *Cancer Res.*, 50:3934-39 (1990); Hoffmann et al., *Anticancer Res.*, 17:4419-26 (1997); Wikstrand et al., *Cancer Res.*, 55:3140-48 (1995), all of which are incorporated by reference.

In vivo assays can also be utilized to detect VEGFR-1 neutralization. For example, receptor tyrosine kinase inhibition can be observed by mitogenic assays using cell lines stimulated with receptor ligand in the presence and absence of inhibitor. For example, HUVEC cells (ATCC) stimulated with VEGF(A) or VEGF-B can be used to assay VEGFR-1 inhibition. Another method involves testing for inhibition of growth of VEGF-expressing tumor cells, using for example, human tumor cells injected into a mouse. *See e.g.*, U.S. Patent No. 6,365,157 (Rockwell et al.), which is incorporated by reference herein.

Of course, the present invention is not limited by any particular mechanism of VEGFR-1 neutralization. Anti-VEGFR-1 antibodies of the present invention can, for example, bind externally to VEGFR-1, block binding of ligand to VEGFR-1 and subsequent signal transduction mediated via receptor-associated tyrosine kinase, and prevent phosphorylation of VEGFR-1 and other downstream proteins in the signal transduction cascade. The receptor-antibody complex can also be internalized and degraded, resulting in receptor cell surface down-regulation. Matrix metalloproteinases, which function is tumor cell invasion and metastasis, can also be down-regulated by anti- VEGFR-1 antibodies of the present invention.

Human anti-VEGFR-1 antibodies can be obtained from naturally occurring antibodies, or Fab or scFv phage display libraries constructed, for example, from human heavy chain and light chain variable region genes and the CDR sequences of the anti-VEGFR-1 antibodies of the present invention can be inserted into such human anti-VEGFR-1 antibodies.

Human anti-VEGFR-1 antibodies can be produced by methods well known to one of skill in the art. Such methods include the hybridoma method using transgenic mice described by Kohler and Milstein, *Nature*, 256: 495-497 (1975) and Campbell, Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas, Burdon *et al.*, Eds., Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985), all of which are incorporated by reference herein; as well as by the recombinant DNA method described by Huse *et al.*, *Science*, 246, 1275-1281 (1989), which is incorporated by reference herein.

Antibody fragments can be produced by cleaving a whole antibody, or by expressing DNA that encodes the fragment. Fragments of antibodies may be prepared by methods described by Lamoyi et al., *J. Immunol. Methods*, 56: 235-243 (1983) and by Parham, *J. Immunol.* 131: 2895-2902 (1983), both of which are incorporated by reference herein. Such fragments may contain one or both Fab fragments or the $F(ab')_2$ fragment. Such fragments may also contain single-chain fragment variable region antibodies, *i.e.* scFv, diabodies, or other antibody fragments. Methods of producing such antibodies are disclosed in PCT Application WO 93/21319, European Patent Application No. 239,400; PCT Application WO 89/09622; European Patent

Application 338,745; and European Patent Application EP 332,424, all of which are incorporated by reference herein.

In another embodiment, the present invention provides polynucleotides encoding the anti-VEGFR-1 antibodies of the present invention. Such polynucleotides encode the light chain CDR2 of SEQ ID NO.: 2, the light chain CDR3 of SEQ ID NO: 3, and, preferably, one or more of the other CDRs listed in Table 1. Table 3 sets forth the nucleic acid sequences of preferred anti-VEGFR-1 antibodies.

Table 3 – Nucleotide sequence of anti-VEGFR-1 antibodies

Clone	Light Chain
6F9	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCCTTGTCTCCAGGGGAA AGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGGTAGCAGCAGCTACTT AGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATG GTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGG TCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTT GCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCGCTCACTTTCGGCGGA GGGACCAAGGTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCAT CTTCCCG SEQ ID NO:21
13G12	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCCTTGTCTCCAGGGGAA AGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGGTAGCAGCAGCTACTT AGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATG GTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGG TCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTT GCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCGCTCACTTTCGGCGGA GGGACCAAGGTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCAT CTTCCCG SEQ ID NO:21
15F11	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAA AGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTT AGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATG GTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGG TCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTT GCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCTCTCACCTTCGGCCAA GGGACACGACTGGAGATTAAACGAACTGTGGCTGCACCATCTGTCTTCAT CTTCCCG SEQ ID NO:22

18F1	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAA AGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTT AGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATG GTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGG TCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTT GCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCGCTCACTTTCGGCGGA GGGACCAAGGTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCAT CTTTCCG SEQ ID NO:23
Clone	Heavy Chain
6F9	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC CCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAGTTATGGCAT GCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTA TATGGTATGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGA TTCACCATCTCCAGAGACAATTCCAAGAACACGGTGTATCTGCAAATGAA CAGCCTGAGAGCCGAGGACACGGCTGTGTATCACTGTACGAGAGATCACT TTGGTTCGGGGGCTCACTACTACTACTACTACGGTATGGACGTCTGGGGCC AAGGGACCACGGTCACCGTCTCCTCA SEQ ID NO:24
13G12	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC CCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTATGGCAT GCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTA TATGGTATGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGA TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAA CAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCACT ATGGTTCGGGGGCTCACTACTACTACTACTACGGTATGGACGTCTGGGGC CAAGGGACCACGGTCACCGTCTCCTCA SEQ ID NO:25
15F11	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC CCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTATGGCAT GCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTA TATGGTTTGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGA TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAA CAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCACT ATGGTTCGGGGGCTCACTCCTACTACTACTACGGTTTGGACGTTTGGGGCC AAGGGACCTCGGTCACCGTCTCCTCA SEQ ID NO:26

18F1	CAGGCGCAGGTGGTGGAGTCTGGGGGAGGCGTGGTCCAGTCTGGGAGGTC CCTGAGACTCTCCTGTGCAGCGTCTGGATTCGCCTTCAGTAGCTACGGCAT GCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTA TATGGTATGATGGAAGTAATAAATACTATGCAGACTCCGTGAGGGGCCGA TTCACCATCTCCAGAGACAATTCCGAGAACACGCTGTATCTGCAAATGAA CAGCCTGAGAGCCGAGGACACCGCTGTGTATTACTGTGCCAGAGATCACT ATGGTTCGGGGGTGCACCACTATTTCTACTACGGTCTGGACGTCTGGGGCC AAGGGACCACGGTCACCGTCTCCTCA SEQ ID NO:27

DNA encoding human antibodies can be prepared by recombining DNA encoding human constant regions and variable regions, other than the CDRs, derived substantially or exclusively from the corresponding human antibody regions and DNA encoding CDRs derived from a human (SEQ ID NOs: 1-4 for the light chain variable domain CDRs and SEQ ID Nos: 5-13 for the heavy chain variable domain CDRs.

Polynucleotides encoding anti-VEGFR-1 antibodies of the present invention include polynucleotides with nucleic acid sequences that are substantially the same as the nucleic acid sequences of the polynucleotides of the present invention. "Substantially the same" nucleic acid sequence is defined herein as a sequence with at least 70%, preferably at least 80%, and more preferably at least 90% identity to another nucleic acid sequence when the two sequences are optimally aligned (with appropriate nucleotide insertions or deletions) and compared to determine exact matches of nucleotides between the two sequences.

Suitable sources of DNAs that encode fragments of antibodies include any cell, such as hybridomas and spleen cells, that express the full-length antibody. The fragments may be used by themselves as antibody equivalents, or may be recombined into equivalents, as described above. The DNA deletions and recombinations described in this section may be carried out by known methods, such as those described in the published patent applications listed above in the section entitled "Functional Equivalents of Antibodies" and/or other standard recombinant DNA techniques, such as those described below. Another source of DNAs are single chain antibodies produced from a phage display library, as is known in the art.

Additionally, the present invention provides expression vectors containing the polynucleotide sequences previously described operably linked to an expression sequence, a promoter and an enhancer sequence. A variety of expression vectors for the efficient synthesis of antibody polypeptide in prokaryotic, such as bacteria and

eukaryotic systems, including but not limited to yeast and mammalian cell culture systems have been developed. The vectors of the present invention can comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences.

Any suitable expression vector can be used. For example, prokaryotic cloning vectors include plasmids from *E. coli*, such as *colE1*, *pCR1*, *pBR322*, *pMB9*, *pUC*, *pKSM*, and *RP4*. Prokaryotic vectors also include derivatives of phage DNA such as *M13* and other filamentous single-stranded DNA phages. An example of a vector useful in yeast is the 2μ plasmid. Suitable vectors for expression in mammalian cells include well-known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and shuttle vectors derived from combination of functional mammalian vectors, such as those described above, and functional plasmids and phage DNA.

Additional eukaryotic expression vectors are known in the art (*e.g.*, P.J. Southern & P. Berg, *J. Mol. Appl. Genet.*, 1:327-341 (1982); Subramani *et al.*, *Mol. Cell. Biol.*, 1: 854-864 (1981); Kaufmann & Sharp, "Amplification And Expression of Sequences Cotransfected with a Modular Dihydrofolate Reductase Complementary DNA Gene," *J. Mol. Biol.*, 159:601-621 (1982); Kaufmann & Sharp, *Mol. Cell. Biol.*, 159:601-664 (1982); Scahill et al., "Expression And Characterization Of The Product Of A Human Immune Interferon DNA Gene In Chinese Hamster Ovary Cells," *Proc. Nat'l Acad. Sci. USA*, 80:4654-4659 (1983); Urlaub & Chasin, *Proc. Nat'l Acad. Sci. USA*, 77:4216-4220, (1980), all of which are incorporated by reference herein).

The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the *lac* system, the *trp* system, the *tac* system, the *trc* system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast, e.g., the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters or SV40, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

The present invention also provides recombinant host cells containing the expression vectors previously described. Anti-VEGFR-1 antibodies of the present invention can be expressed in cell lines other than in hybridomas. Nucleic acids, which comprise a sequence encoding a polypeptide according to the invention, can be used for transformation of a suitable mammalian host cell.

Cell lines of particular preference are selected based on high level of expression, constitutive expression of protein of interest and minimal contamination from host proteins. Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines, such as but not limited to, Chinese Hamster Ovary (CHO) cells, Baby Hamster Kidney (BHK) cells and many others. Suitable additional eukaryotic cells include yeast and other fungi. Useful prokaryotic hosts include, for example, *E. coli*, such as *E. coli* SG-936, *E. coli* HB 101, *E. coli* W3110, *E. coli* X1776, *E. coli* X2282, *E. coli* DHI, and *E. coli* MRCl, *Pseudomonas*, *Bacillus*, such as *Bacillus subtilis*, and *Streptomyces*.

These present recombinant host cells can be used to produce an antibody by culturing the cells under conditions permitting expression of the antibody and purifying the antibody from the host cell or medium surrounding the host cell. Targeting of the expressed antibody for secretion in the recombinant host cells can be facilitated by inserting a signal or secretory leader peptide-encoding sequence (*See*, Shokri *et al.*, (2003) *Appl Microbiol Biotechnol.* 60(6):654-664, Nielsen *et al., Prot. Eng.*, 10:1-6 (1997); von Heinje *et al.*, *Nucl. Acids Res.*, 14:4683-4690 (1986), all of which are incorporated by reference herein) at the 5' end of the antibody-encoding gene of interest. These secretory leader peptide elements can be derived from either prokaryotic or eukaryotic sequences. Accordingly suitably, secretory leader peptides are used, being amino acids joined to the N-terminal end of a polypeptide to direct movement of the polypeptide out of the host cell cytosol and secretion into the medium.

The anti-VEGFR-1 antibodies of the present invention can be fused to additional amino acid residues. Such amino acid residues can be a peptide tag to facilitate isolation, for example. Other amino acid residues for homing of the antibodies to specific organs or tissues are also contemplated.

In another embodiment, the present invention provides methods of treating a medical condition by administering a therapeutically effective amount of an anti-

VEGFR-1 antibody according to the present invention to a mammal in need thereof. Therapeutically effective means an amount effective to produce the desired therapeutic effect, such as inhibiting tyrosine kinase activity.

In a preferred embodiment, the present invention provides a method of reducing tumor growth or inhibiting angiogenesis by administering a therapeutically effective amount of an anti-VEGFR-1 antibody of the present invention to a mammal in need thereof. While not intended to be bound to a particular mechanism, the conditions that may be treated by the present methods include, for example, those in which tumor growth or pathogenic angiogenesis is stimulated through a VEGFR paracrine and/or autocrine loop.

With respect to reducing tumor growth, such tumors include primary tumors and metastatic tumors, as well as refractory tumors. Refractory tumors include tumors that fail to respond or are resistant to other forms of treatment such as treatment with chemotherapeutic agents alone, antibodies alone, radiation alone or combinations thereof. Refractory tumors also encompass tumors that appear to be inhibited by treatment with such agents, but recur up to five years, sometimes up to ten years or longer after treatment is discontinued.

Anti-VEGFR-1 antibodies of the present invention are useful for treating tumors that express VEGFR-1. Such tumors are characteristically sensitive to VEGF present in their environment, and may further produce and be stimulated by VEGF in an autocrine stimulatory loop. The method is therefore effective for treating a solid or non-solid tumor that is not vascularized, or is not yet substantially vascularized.

Examples of solid tumors which may be accordingly treated include breast carcinoma, lung carcinoma, colorectal carcinoma, pancreatic carcinoma, glioma and lymphoma. Some examples of such tumors include epidermoid tumors, squamous tumors, such as head and neck tumors, colorectal tumors, prostate tumors, breast tumors, lung tumors, including small cell and non-small cell lung tumors, pancreatic tumors, thyroid tumors, ovarian tumors, and liver tumors. Other examples include Kaposi's sarcoma, CNS neoplasms, neuroblastomas, capillary hemangioblastomas, meningiomas and cerebral metastases, melanoma, gastrointestinal and renal carcinomas and sarcomas, rhabdomyosarcoma, glioblastoma, preferably glioblastoma multiforme, and leiomyosarcoma. Examples of vascularized skin cancers for which anti-VEGFR-1 antibodies of the present invention are effective include squamous cell

carcinoma, basal cell carcinoma and skin cancers that can be treated by suppressing the growth of malignant keratinocytes, such as human malignant keratinocytes.

Examples of non-solid tumors include leukemia, multiple myeloma and lymphoma. Some examples of leukemias include acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), erythrocytic leukemia or monocytic leukemia. Some examples of lymphomas include Hodgkin's and non-Hodgkin's lymphoma.

With respect to inhibiting angiogenesis, anti-VEGFR-1 antibodies of the present invention are effective for treating subjects with vascularized tumors or neoplasms, or angiogenic diseases characterized by excessive angiogenesis. Such tumors and neoplasms include, for example, malignant tumors and neoplasms, such as blastomas, carcinomas or sarcomas, and highly vascular tumors and neoplasms. Cancers that may be treated by the methods of the present invention include, for example, cancers of the brain, genitourinary tract, lymphatic system, stomach, renal, colon, larynx and lung and bone. Non-limiting examples further include epidermoid tumors, squamous tumors, such as head and neck tumors, colorectal tumors, prostate tumors, breast tumors, lung tumors, including lung adenocarcinoma and small cell and non-small cell lung tumors, pancreatic tumors, thyroid tumors, ovarian tumors, and liver tumors. The method is also used for treatment of vascularized skin cancers, including squamous cell carcinoma, basal cell carcinoma, and skin cancers that can be treated by suppressing the growth of malignant keratinocytes, such as human malignant keratinocytes. Other cancers that can be treated include Kaposi's sarcoma, CNS neoplasms (neuroblastomas, capillary hemangioblastomas, meningiomas and cerebral metastases), melanoma, gastrointestinal and renal carcinomas and sarcomas, rhabdomyosarcoma, glioblastoma, including glioblastoma multiforme, and leiomyosarcoma.

Non-limiting examples of pathological angiogenic conditions characterized by excessive angiogenesis involving, for example inflammation and/or vascularization include atherosclerosis, rheumatoid arthritis (RA), neovascular glaucoma, proliferative retinopathy including proliferative diabetic retinopathy, macular degeneration, hemangiomas, angiofibromas, and psoriasis. Other non-limiting examples of non-neoplastic angiogenic disease are retinopathy of prematurity (retrolental fibroplastic), corneal graft rejection, insulin-dependent diabetes mellitus,

multiple sclerosis, myasthenia gravis, Crohn's disease, autoimmune nephritis, primary biliary cirrhosis, psoriasis, acute pancreatitis, allograph rejection, allergic inflammation, contact dermatitis and delayed hypersensitivity reactions, inflammatory bowel disease, septic shock, osteoporosis, osteoarthritis, cognition defects induced by neuronal inflammation, Osler-Weber syndrome, restinosis, and fungal, parasitic and viral infections, including cytomegaloviral infections.

The identification of medical conditions treatable by anti-VEGFR-1 antibodies of the present invention is well within the ability and knowledge of one skilled in the art. For example, human individuals who are either suffering from a clinically significant neoplastic or angiogenic disease or who are at risk of developing clinically significant symptoms are suitable for administration of the present VEGF receptor antibodies. A clinician skilled in the art can readily determine, for example, by the use of clinical tests, physical examination and medical/family history, if an individual is a candidate for such treatment.

Anti-VEGFR-1 antibodies of the present invention can be administered for therapeutic treatments to a patient suffering from a tumor or angiogenesis associated pathologic condition in an amount sufficient to prevent, inhibit, or reduce the progression of the tumor or pathologic condition. Progression includes, *e.g*, the growth, invasiveness, metastases and/or recurrence of the tumor or pathologic condition. Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's own immune system. Dosing schedules will also vary with the disease state and status of the patient, and will typically range from a single bolus dosage or continuous infusion to multiple administrations per day (e.g., every 4-6 hours), or as indicated by the treating physician and the patient's condition. It should be noted, however, that the present invention is not limited to any particular dose.

In another embodiment, the present invention provides a method of treating a medical condition by administering an anti-VEGFR-1 antibody of the present invention in combination with one or more other agents. For example, an embodiment of the present invention provides a method of treating a medical condition by administering an anti-VEGFR-1 antibody of the present invention with an antineoplastic or antiangiogenic agent. The anti-VEGFR-1 antibody can be

chemically or biosynthetically linked to one or more of the antineoplastic or antiangiogenic agents.

Any suitable antineoplastic agent can be used, such as a chemotherapeutic agent or radiation. Examples of chemotherapeutic agents include, but are not limited to, cisplatin, doxorubicin, cyclophosphamide, paclitaxel, irinotecan (CPT-11), topotecan or a combination thereof. When the antineoplastic agent is radiation, the source of the radiation can be either external (external beam radiation therapy – EBRT) or internal (brachytherapy – BT) to the patient being treated.

Further, anti-VEGFR-1 antibodies of the present invention may be administered with antibodies that neutralize other receptors involved in tumor growth or angiogenesis. One example of such a receptor is VEGFR-2/KDR. In an embodiment, an anti-VEGR-1 antibody of the present invention is used in combination with a receptor antagonist that binds specifically to VEGFR-2. Particularly preferred are antigen-binding proteins that bind to the extracellular domain of VEGFR-2 and block binding by any one of its ligands, such as VEGF(A), VEGF-C, VEGF-D, or VEGF-E.

Another example of such a receptor is EGFR. In an embodiment of the present invention, an anti-VEGFR-1 antibody is used in combination with an EGFR antagonist. An EGFR antagonist can be an antibody that binds to EGFR or a ligand of EGFR and inhibits binding of EGFR to its ligand. Ligands for EGFR include, for example, EGF, TGF-α amphiregulin, heparin-binding EGF (HB-EGF) and betarecullulin. EGF and TGF-α are thought to be the main endogenous ligands that result in EGFR-mediated stimulation, although TGF-α has been shown to be more potent in promoting angiogenesis. It should be appreciated that the EGFR antagonist can bind externally to the extracellular portion of EGFR, which may or may not inhibit binding of the ligand, or internally to the tyrosine kinase domain. Examples of EGFR antagonists that bind EGFR include, without limitation, biological molecules, such as antibodies (and functional equivalents thereof) specific for EGFR, and small molecules, such as synthetic kinase inhibitors that act directly on the cytoplasmic domain of EGFR.

Other examples of growth factor receptors involved in tumorigenesis are the receptors for platelet-derived growth factor (PDGFR), insulin-like growth factor (IGFR), nerve growth factor (NGFR), and fibroblast growth factor (FGFR).

In an additional alternative embodiment, the present invention provides a method of treating a medical condition by administering an anti-VEGFR-1 antibody of the present invention in combination with one or more suitable adjuvants, such as, for example, cytokines (IL-10 and IL-13, for example) or other immune stimulators. *See, e.g.*, Larrivée *et al., supra.*

In a combination therapy, the anti-VEGFR-1 antibody can be administered before, during, or after commencing therapy with another agent, as well as any combination thereof, *i.e.*, before and during, before and after, during and after, or before, during and after commencing the antineoplastic agent therapy. For example, an anti-VEGFR-1 antibody of the present invention may be administered between 1 and 30 days, preferably 3 and 20 days, more preferably between 5 and 12 days before commencing radiation therapy. The present invention, however is not limited to any particular administration schedule. The dose of the other agent administered depends on numerous factors, including, for example, the type of agent, the type and severity of the medical condition being treated and the route of administration of the agent. The present invention, however, is not limited to any particular dose.

Any suitable method or route can be used to administer an anti-VEGFR-1 antibody of the present invention, and optionally, to coadminister antineoplastic agents and/or antagonists of other receptors. Routes of administration include, for example, oral, intravenous, intraperitoneal, subcutaneous, or intramuscular administration. It should be emphasized, however, that the present invention is not limited to any particular method or route of administration.

It is noted that an anti-VEGFR-1 antibody of the present invention can be administered as a conjugate, which binds specifically to the receptor and delivers a toxic, lethal payload following ligand-toxin internalization.

It is understood that anti-VEGFR-1 antibodies of the invention, where used in a mammal for the purpose of prophylaxis or treatment, will be administered in the form of a composition additionally comprising a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers include, for example, one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the

binding proteins. The compositions of the injection may, as is well known in the art, be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the mammal.

Although human antibodies of the invention are particularly useful for administration to humans, they may be administered to other mammals as well. The term "mammal" as used herein is intended to include, but is not limited to, humans, laboratory animals, domestic pets and farm animals.

The present invention also includes kits for inhibiting tumor growth and/or angiogenesis comprising a therapeutically effective amount of an anti-VEGFR-1 antibody of the present invention. The kits can further contain any suitable antagonist of, for example, another growth factor receptor involved in tumorigenesis or angiogenesis (e.g., VEGFR-2/FKDR, EGFR, PDGFR, IGFR, NGFR, FGFR, etc, as described above). Alternatively, or in addition, the kits of the present invention can further comprise an antineoplastic agent. Examples of suitable antineoplastic agents in the context of the present invention have been described herein. The kits of the present invention can further comprise an adjuvant, examples of which have also been described above.

In another embodiment, the present invention provides investigative or diagnostic methods using anti-VEGFR-1 antibodies of the present invention *in vivo* or *in vitro*. In such methods, anti-VEGFR-1 antibodies can be linked to target or reporter moieties.

EXAMPLES

The following examples do not include detailed descriptions of conventional methods, such as those employed in the construction of vectors and plasmids, the insertion of genes encoding polypeptides into such vectors and plasmids, or the introduction of plasmids into host cells. Such methods are well known to those of ordinary skill in the art and are described in numerous publications including Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989), Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, which is incorporated by reference herein.

Materials

All reagents and chemicals were purchased from Sigma (St. Louis, MO) unless otherwise noted. Human VEGF165 and soluble recombinant human VEGFR-1 alkaline phosphatase (rhuVEGFR-1 AP) proteins were expressed in stably transfected cells and purified from cell culture supernatant following the procedures known to one skilled in the art (Tessler, *J. Biol. Chem.*, 269:12456-12461 (1994), which is incorporated by reference herein). PlGF and soluble recombinant VEGFR-1 Fc (rhuVEGFR-1 Fc) proteins were purchased from (R&D Systems Inc. Minneapolis, MN). Cell cultureware and assay plates were purchased from (BD Biosciences, Bedford, MA).

Cell Lines

The human breast cancer cell lines DU4475, MDA-MB-231, MDA-MB-435, and mouse myeloma cell lines P3-X63-Ag8.653 and NS0 were obtained from American Type Tissue Culture Collection (Manassas, VA). P3-X63-Ag8.653 Bcl/2 transfectant cell line was created in house as previously described (Ray S, Diamond B. Proc Natl Acad Sci USA. 91:5548-51, 1994). The tumor cells were maintained in RPMI1640 medium (Invitrogen/Life Technologies, Inc., Rockville, MD) containing 10% FCS (Hyclone, Logan, UT). Porcine aorta endothelial VEGFR-1 expressing cell line was provided by Dr. L. Claesson-Welsh, Uppsala University, and cultured in F12 medium (Invitrogen/Life Technologies, Inc., Rockville, MD) containing 10% FCS (Hyclone, Logan, UT). All cells were maintained at 37°C in a humidified, 5% CO2 atmosphere.

Example 1: Generation of anti-VEGFR 1 antibodies

Human anti-VEGFR-1 monoclonal antibodies (referred to herein as "anti-VEGFR-1 antibodies") were generated by a standard hybridoma technology (Harlow & Lane, ed., Antibodies: A Laboratory Manual, Cold Spring Harbor, 211-213 (1998), which is incorporated by reference herein) using KM transgenic mice (Medarex, San Jose, Calif.), which produce human immunoglobulin gamma heavy and kappa light chains. KM mice were immunized subcutaneously (s.c.) with VEGFR-1 fragment crystallization (Fc) in complete Freund's adjuvant. Animals were intraperitoneally (i.p.) boosted three times with the same VEGFR-1 protein in incomplete Freund's adjuvant before fusion. The animals were rested for a month before they received the final i.p. boost of 25 micrograms of VEGFR-1 protein in phosphate buffer solution

(PBS). Four days later, splenocytes were harvested from the immunized mouse and fused with P3-X63-Ag8.653 Bcl-2 transfectant plasmacytoma cells using polyethylene glycol (PEG, MW: 1450 KD). After fusion, the cells were resuspended in HAT (hypoxanthine, aminopterin, thymidine) medium supplemented with 10% fetal bovine serum (FBS) and distributed to 96 well plates at a density of 200 microliters per well for establishment of hybridoma cells. At day 6 post-fusion, 100 microliters of medium was aspirated and replaced with 100 microliters of fresh medium.

Example 2A: Anti-VEGFR-1 Antibodies From Example 1 Bind to VEGFR-1 and Inhibit VEGFR-1 Binding to Its Ligands

a. VEGFR-1 Binding and Blocking Assays

At day 10-12 post-fusion, the hybridomas were screened for antibody production and specific binding activity of culture supernatant with rhuVEGFR-1 protein in ELISA-based binding and blocking assays. The positive hybridomas were subcloned three times by a limiting dilution culture for establishment of monoclonal hybridomas.

Specifically, hybridoma supernatants or purified antibodies were diluted in PBS with 5% FBS and 0.05% Tween 20 (ELISA buffer) and incubated in rhuVEGFR-1 AP or AP coated 96-well microtiter plates for 30 minutes. Plates were washed with the ELISA buffer and incubated with goat anti-mouse IgG-horseradish peroxidase (HRP) conjugate (BioSource International, Camarillo, CA) for 30 minutes. TMB (3,3', 5,5'-tetra-methylbenzidine) substrate (Kierkegaard and Perry Lab, Inc., Gaithersburg, MD) was used for color development following the manufacturer's instruction. The absorbance at 450 nanometers (nm) was read for quantification of binding activity of antibodies. For identification of the hybridomas producing anti-VEGFR-1 antibodies, hybridoma supernatants were preincubated with VEGFR-1 AP for 1 hour. The mixtures were incubated with the ELISA buffer in VEGF or PlGF coated 96-well microtiter plates for 1 hour. PNPP (p-nitrophenyl phosphate) substrate for AP was used for color development following the manufacturer's instruction. The absorbance at 405 nm was read for quantification of VEGFR-1 binding to VEGF or PlGF. Optical density (OD) values were read on a microtiter plate reader (Molecular

Devices Corp., Sunnyvale, CA). ED50 and IC50 of the antibodies were analyzed using GraphPad Prism 3 software (GraphPad Software, Inc., San Diego, CA).

Figure 3 shows the binding activity of purified antibodies produced from hybridomas designated "6F9," "13G12," "15F11," and "18F1." These antibodies exhibited a binding activity with ED50 of 0.1-0.3 nM in ELISA-based binding assay. **Figures 4** and **5** show respectively that clones 6F9, 13G12, 15F11, 18F1 effectively blocked PIGF binding to VEGFR-1 with IC50 of 0.4-0.8 nM and VEGF binding to VEGFR-1 with IC50 of 0.7-0.8 nM. The binding and blocking characteristics of the antibodies are summarized in Table 4.

Table 4 – Binding and Blocking Characteristics of anti-VEGFR-1 antibodies

Clone	Binding Activity (ED50)	Blocking Activity (IC50)
6F9	0.1 nM	0.86 nM: PlGF 0.82 nM: VEGF
13G12	0.3 nM	0.82 nM: PlGF 0.70 nM: VEGF
15F11	0.3 nM	0.49 nM: PlGF 0.73 nM: VEGF
18F1	0.1 nM	0.55 nM: PlGF 0.84 nM: VEGF

b. Measurement of Affinity of Anti-VEGFR-1 Antibodies

Affinities of anti-VEGFR-1 antibody clones 6F9, 13G12, 15F11, 18F1 were determined by plasmon resonance technology using BIAcore 2000 (Pharmacia, Piscataway, NJ) according to the procedures provided by the manufacturer. Kinetic analyses of the antibodies were performed by immobilization of recombinant extracellular domain of VEGFR-1 onto a sensor surface at a low density. The (k_{on})

and dissociation (k_{off}) rates were determined using the BIAevaluation 2.1 software provided by the manufacturer.

Anti-VEGFR-1 antibody clones 6F9, 13G12, F11, and 18F1 exhibited a high affinity with a K_D value of 69, 121, 70, and 54 pM, respectively. The kinetics of the antibodies are summarized in Table 5.

Table 5 – Kinetics of human anti-VEGFR-1 antibodies

Clone	K_{on}	K_{off}	K_D
6F9	1.01e6 M	7.38e-5 M	69 pM
13G12	0.95e6 M	10.9e-5 M	121 pM
15F11	1.02e6 M	7.16e-5 M	70 pM
18F1	0.81e6 M	4.27e-5 M	54 pM

c. Evaluation of Specificity of Anti-VEGFR-1 Antibody

To determine the specificity of an anti-VEGFR-1 monoclonal antibody to human VEGFR-1, purified antibodies 18F1 were tested in an ELISA-based assay. One μg/ml of recombinant human VEGFR-1 Fc, mouse VEGFR-1 Fc, mouse VEGFR-2 Fc, or human VEGFR-2 alkaline phosphatase was coated with PBS in a 96-well microtiter plates at 4 °C over night. After wash, the receptor coated plates were blocked with PBS containing 5% Dry Milk and 0.05% Tween 20. Serial dilutions of primary antibody 18F1 to human VEGFR-1, MF1 to mouse VEGFR-1, 1C11 to human VEGFR-2, or DC101 to mouse VEGFR-2 were incubated in the receptor-coated plates for 30 minutes. After wash secondary anti-primary HRP conjugate antibodies was incubated in the plates for 30 minutes. Plates were washed and incubated with the substrate TMB (3,3', 5,5'-tetra-methylbenzidine) for color development. The absorbance at 450 nm was read as OD values for quantification of binding activity of antibodies. Data were analyzed using a GraphPad Prism Software.

Figures 6 A-D show the specificity of monoclonal antibody 18F1 to human VEGFR-1 (**Figure 6A**), and that the antibody has no cross reactivity with mouse

VEGFR-1 (**Figure 6B**), human VEGFR-2 (**Figure 6C**) and mouse VEGFR-2 (**Figure 6D**). The results indicate that the anti-human VEGFR-1 antibody 18F1 has a strict binding specificity with its respective receptor.

d. Western Blot

Confluent porcine aorta endothelial VEGFR-1 expressing (PAE-VEGFR-1) cells and BT474 human breast carcinoma cells were cultured in serum-depleted F12 medium for 48 hours. The cells were then preincubated with anti-VEGFR-1 antibody clone 18F1 at concentrations ranging from 0.1 to 30 μg/ml for 1 hour followed by stimulating with VEGF or PlGF for 5 minutes at 37°C. The cells were then rinsed with ice-cold PBS and lysed in lysis buffer (50 mM HEPES, 150 mM NaCl, 1% Triton X-100, and 10% glycerol containing 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM sodium vanadate). Cell lysates were subjected to SDS-PAGE and transferred onto Immobilon membranes (Millipore Corp. Billerica, MA). After transfer, blots were incubated with the blocking solution and probed with antiphosphotyrosine antibody (PY20, Santa Cruz Biotechnology, Santa Cruz, CA) followed by washing. The protein contents were visualized using horseradish peroxidase-conjugated secondary antibodies followed by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ). An anti-VEGFR-1 specific antibody (Oncogene Research Products, San Diego, CA) was used for re-blot of VEGFR-1.

All anti-VEGFR-1 antibodies recognized a 180 KD molecule of VEGFR-1 recombinant protein.

Example 2B: Anti-Human Anti-VEGFR-1 Antibody is Specific for Human VEGFR-1

HuVEGFR-1-Fc, mouse VEGFR-1-AP (ImClone Systems) or huVEGFR-2-AP (ImClone Systems) (100 ng/well) was coated on 96 strip-well plates and blocked with 5% milk/PBS. The binding of 18F1 and other anti-human VEGFR-1 antibodies or a rat anti-mouse VEGFR-1 antibody, MF1 (ImClone Systems, ref. 18), to plate bound VEGFR-1 or VEGFR-2 was evaluated as described for the hybridoma supernatant screening above, except that bound antibody was detected with a goat anti-human kappa-HRP antibody (BioSource International, Camarillo, CA) for 18F1

and anti-human VEGFR-2 antibody 1C11, or a goat anti-rat IgG-HRP antibody (BioSource International) for MF1.

8F1 showed a specific reactivity with human VEGFR-1 (**Figure 32A**) but no cross reactivity with mouse VEGFR-1 (**Figure 32B**) and human VEGFR-2 (**Figure 32C**). The anti-mouse VEGFR-1 blocking antibody MF1 was also demonstrated to be species specific, binding mouse (**Figure 32B**). but not human VEGFR-1 (**Figure 32A**).

Example 3: Anti-VEGFR-1 Antibodies Bind to Native VEGFR-1 on VEGFR-1 Expressing Cells

a. Flow Cytometry Analysis

Aliquots of 10^6 PAE-VEGFR-1 cells were harvested from subconfluent cultures and incubated with anti-VEGFR-1 antibody clones 6F9, 13G12, F11, and 18F1 in PBS with 1% bovine serum albumin (BSA) and 0.02% sodium azide (staining buffer) for one hour on ice. Aliquots of 10^6 DU4475 human breast carcinoma cells were harvested from subconfluent cultures and incubated with anti-VEGFR-1 antibody clone 18F1 in PBS with 1% bovine serum albumin (BSA) and 0.02% sodium azide (staining buffer) for one hour on ice. A matched IgG isotype (Jackson ImmunoResearch, West Grove, PA) was used as a negative control. Cells were washed twice with flow buffer and then incubated with a fluorescein isothiocyanate (FITC)-labeled goat anti-human IgG antibody (BioSource International, Camarillo, CA) in staining buffer for 30 minutes on ice. Cells were washed as above and analyzed on an Epics XL flow cytometer (Beckman-Coulter, Hialeah, FL). Dead cells and debris were eliminated from the analysis on the basis of forward and sideways light scatter. The mean fluorescent intensity units (MFIU) were calculated as the mean log fluorescence multiplied by the percentage of positive population.

Figure 7 shows binding reactivity of clones 6F9, 13G12, 15F11 and 18F1 with the PAE-VEGFR-1 expressing cells. **Figures 8A** and **8B** show binding reactivity of clone 18F1 with PAE-VEGFR-1 expressing cells and DU4475 human breast carcinoma, respectively. These results indicate that the human anti-VEGFR-1 antibodies bind to native VEGFR-1 expressed in cell surface.

b. Surface VEGFR-1 blocking assay

The binding of ^{125}I-VEGF to VEGFR-1 on cell surface was performed using PAE-VEGFR-1 expressing cells. Cells were grown on non-coated plastic cell culture plates, which were found to decrease nonspecific binding without affecting the specific binding of ^{125}I-VEGF. Confluent cells were incubated in serum- and growth supplement-free Dulbecco's Modified Eagle Medium (DMEM)/F-12 medium (Invitrogen, Carlsbad, CA) for 24 hours. Cells were rinsed once with ice-cold DMEM/F-12 medium containing 0.025 M HEPES and 1 mg/ml bovine serum albumin (BSA). A serial dilution of anti-VEGFR-1 antibody 18F1 or cold VEGF at the concentration of a 200-fold molar excess of labeled VEGF was added to each well in the plate and incubated at 4 °C for 1 hour. After wash, ^{125}I-VEGF was added at the concentration of 2 ng/ml and was incubated at 4 °C for 2 hours on a platform shaker. The cells were washed three times with PBS containing 1 mg/ml BSA and 0.25 mM $CaCl_2$, and were incubated for 5 minutes in the presence of 1% Triton X-100, 1 mg/ml BSA, and 0.16% NaN_3 to remove bound VEGF. The soluble content of each well was counted in a gamma counter. The assays were performed in triplicate in at least three independent experiments and the data were analyzed using Prism GraphPad software 3.03.

Figure 9 shows the strong blocking activity of the anti-VEGFR-1 antibody 18F1 that dramatically prevents the native VEGFR-1 from binding to the ^{125}I-VEGF on the porcine aorta endothelial cells.

Example 4: Anti-VEGFR-1 Antibodies Inhibit Autophosphorylation of VEGFR-1 and Activation of MAPK and Akt in Response to VEGF and PIGF

a. VEGFR-1 Phosphorylation Assay

Autophosphorylation of the VEGFR-1 induced by its ligands and resulting activation of a classical MAPK, extracellular signal-regulated protein kinases 1/2 (ERK1/2) and the PI3K/Atk downstrean signaling pathways mediate cellular biological responses such as proliferation, motility, survival, and differentiation. The ability of an anti-VEGFR-1 antibody to inhibit phosphorylation of VEGFR-1 and activation of ERK1/2 and the Akt kinases downstream signaling were determined by using the PAE-VEGFR-1 transfectant and BT474 breast carcinoma cells.

PAE-VEGFR-1 and BT474 cells were seeded at a density of 5 x 10^5/well in 100 or 150 mm^2 plates and cultured in serum-free medium for 18-48 hours. After

replacing the culture medium, the cells were treated at 37 °C with anti-VEGFR-1 antibody clones 6F9, 15F11,and 18F1 or isotype control for 1 hour and then incubated with 50 ng/ml of VEGF or 100 ng/ml of PlGF for 10 minutes. After treatments, total cell protein extracts were isolated with lysis buffer [20 mM HEPES (pH 7.4), 10 mM $MgCl_2$, 2 mM, $MnCl_2$, 0.05% Triton X-100, and 1 mM DTT], and immunoprecipitated with anti-VEGFR-1 antibody (C-17, Santa Cruz Biotechnology, Santa Cruz, CA). Western blot of phosphorylated VEGFR-1 was detected using anti-phospho-kinase antibody (PY-20, Santa Cruz Biotechnology, Santa Cruz, CA). Proteins were detected using and electrogenerated chemiluminescence system (ECL)(Amersham Pharmacia Biotech, Piscataway, NJ), and quantified by densitometry using NIH Image (National Institute of Mental Health, Bethesda, MD).

b. In vitro Kinase Assay

For evaluation of MAPK and Akt phosphorylation, BT474 cells were seeded at a density of 5 x 10^5/well in 12 well plates in serum-free conditions for 18 hours. Cells were treated at 37 °C with anti-VEGFR-1 antibody clone 18F1 or isotype control for 1 hour and then incubated with 50 ng/ml of VEGF or 100 ng/ml of PlGF for 5-10 minutes. Cell lysis, protein isolation and electroblotting were performed. Membranes were incubated with antibodies against phosphorylated p44/p42 MAP kinases (Thr202/Tyr204, Santa Cruz Biotechnology, Santa Cruz, CA) or phosphorylated Akt (Ser473, Cell Signaling Technology, Beverly, MA), at a concentration of 1 μg/ml, followed by incubation with a secondary IgG-HRP (1:5000). To ensure equal loading of samples, membranes were stripped and reprobed with anti-p44/p42 (Santa Cruz Biotechnology, Santa Cruz, CA) or anti-Akt antibodies (Cell Signaling Technology, Beverly, MA).

c. Results

As shown in **Figures 10-14** a significant phosphorylation of VEGFR-1 and activation of ERK1/2 and Akt signaling in the PAE-VEGFR-1 transfectant and BT474 breast cancer cells was induced by VEGF and PlGF stimulation, suggesting the intrinsic activity of the VEGFR-1 and the receptor-associated downstream kinase signaling pathways in both breast cancer and endothelial cells. As shown in **Figures 10** and **11**, respectively, treatment with anti-VEGFR-1 antibody 18F1 significantly

reduced PlGF or VEGF-stimulated phosphorylation of VEGFR-1 compared to untreated control in PAE-VEGFR-1 transfectant and BT474 breast cancer cells. As shown in **Figures 12** and **13** respectively, treatment with anti-VEGFR-1 antibodies 15F11 and 6F9 also dramatically inhibited PlGF and VEGF induced activation of ERK1/2 downstream signaling induced by PlGF and VEGF in PAE-VEGFR-1 transfectant cells. Activation of Akt protein kinase is an important intracellular signaling event mediating cell survival in breast cancer. As shown in **Figure 14A** and **B**, respectively, treatment with anti-VEGFR-1 antibody 18F1 dramatically inhibited PIGF or VEGF-induced activation of ERK ½ downstream signaling induced by PIGF and VEGF in PAE-VEGFR-1 transfectant cells.

As shown in **Figure 15,** the anti-VEGFR-1 antibody 18F1 significantly blocked PlGF-stimulated phosphorylation of Akt in BT474 breast cancer cells. These results demonstrated that treatment with the anti-VEGFR-1 antibodies is effective to inhibit activation of the VEGFR-1 and downstream signaling kinase pathways in both breast cancer and endothelial cells.

Example 5: Anti-VEGFR-1 Antibodies Blocks *In Vitro* Growth of Breast Tumor Cells

Tumor hypoxia is associated with enhancement of malignant progression, increase of aggressiveness and chemotherapeutic drug resistance. Hypoxic tumor cells undergo biological responses that activate signaling pathways for survival and proliferation by upregulation of a variety of gene expression including the VEGFR-1 (Harris AL. Nat Rev Cancer. 2:38-47, 2002).

Cell Growth Assay

DU4475 carcinoma cells were seeded at a density of 5 x 10^3/well into 96-well plates in serum-free conditions for 18 hours, and in some case followed by treatment with 100 nM of desferrioxamine for additional 5 hours. Inhibitory effect of anti-VEGFR-1 antibody on tumor cell growth was determined by incubation of cells with anti-VEGFR-1 antibody clones 6F9, 13G12, 15F11, and 18F1 at doses of 3, 10, and 30 μg/ml in the presence of 50 ng/ml of VEGF or 200 ng/ml of PlGF for 48 hours. Viable cells were then counted in triplicate using a Coulter cytometer (Coulter Electronics Ltd. Luton, Beds, England). Each experiment was done in triplicate.

The growth rate of the hypoxia-mimic agent desferrioxamine pre-treated DU4475 tumor cells was increased by approximately 2 fold in response to either VEGF or PlGF stimulation. As shown in **Figures 16** and **17**, respectively, treatment with the anti-VEGFR-1 antibodies effectively reduced VEGF and PlGF stimulated proliferation of DU4475 breast carcinoma cells in a dose response manner. **Figure 18A** and **B** separately plots the antibody concentration of antibody clone 18F1 versus cell count of VEGF and PIGF stimulated proliferation of DU4475 breast carcinoma cells. The inhibition of PIGF-induced DU4475 cell growth *in vitro* by the anti-VEGFR-1 antibodies as represented in IC50 values is summarized in Table 6.

Table 6 – Inhibition of PlGF-induced DU4475 cell growth in vitro	
Clone	*in vitro* cell growth
6F9	IC50: 43nM
13G12	IC50: 66nM
15F11	IC50: 44nM
18F1	IC50: 24Nm

Example 6A: Anti-VEGFR-1 Antibodies Suppress Growth of Breast Tumor Xenografts

Treatment of Human Breast Carcinoma Xenografts

Antitumor efficacy of the human anti-VEGFR-1 antibodies was tested in the human xenograft breast tumor models.

Athymic nude mice (Charles River Laboratories, Wilmington, MA) were injected subcutaneously in the left flank area with 2 x 10^6 of DU4475 cells or 5 x 10^6 of MDA-MB-231 and MDA-MB-435 cells mixed in Matrigel (Collaborative Research Biochemicals, Bedford, MA). In the DU4475 and MDA-MB-231 models, tumors were allowed to reach approximately 200 mm^3 in size and then mice were randomized into groups of 12-16 animals per group. Animals received i.p. administration of the anti-VEGFR-1 antibody clones 6F9, 15F11, or 18F1 at a dose of 0.5 mg (MDA-MB-

231) or 1 mg (DU4475) three times each week In the MDA-MB-435 model, the tumor cells were implanted subcutaneously into mammary fat pad area in the mouse. After tumors grew to reach approximately 200 mm^3 in size, mice were randomized into groups of 15 animals per group and intraperitoneally administered with 0.5 mg per dose of 18F1 antibody three times each week. Mice in control groups received an equal volume of saline solution. Treatment of animals was continued for the duration of the experiment. Tumors were measured twice each week with calipers. Tumor volumes were calculated using the formula [π/6 (w1 X w2 X w2)], where "w1" represents the largest tumor diameter and "w2" represents the smallest tumor diameter.

As shown in **Figure 19A** and **19B**, systemic administration of anti-VEGFR-1 antibodies 6F9, 15F11, 13G12 and 18F1 at a dose of 1 mg per dose three times each week led to a statistically significant suppression of tumor growth of the DU4475 xenograft ($p<0.05$). As shown in **Figures 20 A, B**, and **C** respectively, systemic administration of anti-VEGFR-1 antibody 18F1 at a dose of 0.5 or 1 mg per dose three times each week led to a statistically significant suppression of tumor growth of the DU4475, MDA-MB-231, MDA-MB-435 xenografts (ANOVA $p<0.05$). As shown in **Figures 21A** and **B**, treatment with antibody clone 18F1 against human VEGFR-1 for inhibiting cancer cell growth and clone MF1 against mouse VEGFR-1 for inhibiting tumor angiogenesis at a dose of 20 or 40 mg/kg twice each week resulted in a stronger inhibition of tumor growth in the DU4475 and MDA-MB-231 xenograft models ($P<0.05$) when compared to either antibody alone. These results demonstrate that blockade of the *in vivo* function of VEGFR-1 in directly promoting cancer cell growth and modulating tumor vascularization by the anti-VEGFR-1 antibody is effective to suppress growth of VEGFR-1 positive breast tumors in xenograft models.

Example 6B: Anti-Human Anti-VEGFR-1 Antibody Blocks *In Vitro* Growth of Breast Cancer Cells

DU4475 carcinoma cells (2×10^4 per well) were seeded into 24-well plates in serum-free conditions for 18 hours and then treated with hypoxia-mimic agent desferrioxamine (Sigma) for an additional 6 hours. A serial dilution of anti-human VEGFR-1 antibody 18F1 was added to the plates in triplicate and incubated in the

presence of 50 ng/mL of VEGF-A (R&D Systems) or 200 ng/mL of PlGF for 48 hours. Total cell number (bound and in suspension) was determined for each well using a Coulter cell counter (Coulter Electronics Ltd., England).

Treatment of IMC-18F1 significantly blocked VEGF-A and PlGF stimulated proliferation of DU4475 breast carcinoma cells (**Figure 31A and 31B**, respectively; estimated IC50:30-50 nM). The isotype control antibody had no effect on cell proliferation. Thus, 18F1 inhibited VEGFR-1 ligand induced promotion of tumor cell proliferation/survival.

Example 7: Anti-VEGFR-1 Antibody Inhibits VEGF-A and VEGF-B Stimulated Colony Formation of Colon Cancer Cells

One mL DMEM medium containing 10% FBS and 1% agarose (Cambrex Corporation, East Rutherford, NJ) was plated in each well of six well plates. HT-29 human colon carcinoma cells in serum free medium were treated with 66 nM 18F1 or control IgG for 1 hour and followed by treatment with 10 ng/mL VEGF-A or 50 ng/mL VEGF-B for additional 4 hours. The treated cells were mixed with 1 mL 10% FBS DMEM containing 0.5 % agarose and the appropriate antibodies and/or ligands. One mL of this suspension, containing 250 cells, was plated in each well on top of the 1% agarose base layer. After 2 days, additional medium containing antibodies and/or ligands was added to the wells to keep the agarose hydrated. Cells were allowed to grow for 14 days at 37°C. Afterwards, colonies larger than 50 μm in diameter were counted using a dissecting microscope. Statistical analysis was performed using InStat Statistical Software (V2.03, GraphPad Software, San Diego, CA)

The number and size of colonies were significantly increased in the wells where cells were treated with VEGF-A or VEGF-B compared to untreated cells in complete medium only. As shown in **Figure 22**, treatment with 18F1 completely suppressed ligand-induced colony formation compared to basal activity in the absence of stimulation with ligands ($p < 0.03$) (Figure 22). Thus, for both adherent and non-adherent cells, 18F1 has the capability of suppressing the survival and growth of tumor cells.

Example 8: Anti-VEGFR-1 Antibody Inhibits VEGF-A and VEGF-B Induced Migration and Invasion of Colon Cancer Cells

HT-29 cells (2.5×10^4) or SW480 cells (1.5×10^4) were incubated in medium containing 1% FBS with the anti-VEGFR-1 antibody 18F1 (66 nM) in the upper surface of a 24 well MATRIGEL™ coated (HT-29) or uncoated (SW480) 8.0-μm pore size membrane insert (Becton Dickinson Labware, Bedford, MA). The inserts were placed into lower chambers containing 10 ng/mL VEGF-A (R&D Systems) or 50 ng/mL VEGF-B (R&D Systems) for 48 hours. Cells remaining in the top chamber of the inserts were removed with a cotton swab. Cells migrating to the underside of the inserts were stained with Diff-Quik (Harleco, Gibbstown, NJ) and counted in ten random fields at 100X magnification. Statistical analysis was performed using InStat Statistical Software (V2.03, GraphPad Software, San Diego, CA).

As shown in **Figure 23A** and **23B**, VEGF-A or VEGF-B induced migration of HT-29 cells towards ligand through an uncoated membrane. As shown in **Figure 24A** and **24B**, these ligands also induced invasion of SW480 cells through a MATRIGEL™ coated membrane. 18F1 completely blocked VEGFR-1 ligand induced migration and invasion compared to basal activity in the absence of stimulation with ligands ($p < 0.05$, **Figures 23 and 24**). Thus, in addition to negative effects on tumor cell proliferation and survival, 18F1 may provide a means to inhibit the invasion and subsequent metastasis of tumor cells.

Example 9: Treatment with Anti-VEGFR-1 Specific Antibody Suppresses *In Vivo* Growth of VEGFR-1 Expressing Human Xenograft Tumors

Female athymic nu/nu mice, 6-8 weeks of age, were injected subcutaneously on the lateral dorsal surface with 0.4 mL volume of a suspension containing a human tumor cell line in media, diluted 1:1 with MATRIGEL™ (BD Biosciences). The cell lines used in xenograft models, with the cell doses indicated in parenthesis (10^6 cells/mouse), were: human colon carcinoma cell lines DLD-1 (5), GEO (5) and HT-29 (5); human breast carcinoma cell lines DU4475 (2), MDA-MB-231 (5), MDA-MB-435 (5), and BT474 (5). When tumors reached approximately 200-300 mm^3, mice were randomized by tumor size and divided into treatment groups. Tumor growth was evaluated approximately twice weekly, with tumor volume calculated as $\pi/6$ * (Length * $Width^2$), where Length = longest diameter and Width = diameter perpendicular to Length. Tumor dimensions were measured with calipers. T/C% was

calculated as 100 * (Final Treatment Tumor Volume/Initial Treatment Tumor Volume) / (Final Control Tumor Volume/Initial Control Tumor Volume).

18F1 was diluted in 0.9% USP saline (Braun) or phosphate buffered saline (PBS) and administered intraperitoneally in a volume of 0.5 mL per mouse. The effect of treatment on tumor growth was analyzed using repeated measures analysis of variance (RM ANOVA), $p<0.05$ was considered significant.

As shown in **Figure 25**, administration of intraperitoneal 18F1 significantly ($p<0.05$) suppressed the growth of DU4475 (**Figure 25A**), MDA-MB-231, and MDA-MB-435 (**Figure 25B**) xenograft tumors. As shown in **Figure 26**, a significant antitumor effect of 18F1 monotherapy was also observed against HT-29 (**Figure 26A**), DLD-1 (**Figure 26B**), and GEO (**Figure 26C**) colon cancer xenografts. These results demonstrate that blockade of human VEGFR-1 effectively suppresses the growth of xenograft tumors established with VEGFR-1 expressing human tumor cell lines.

Example 10: Anti-Human VEGFR-1 Treatment Inhibits *In Vivo* Signaling of Proliferation and Survival Pathways and Induced Tumor Cell Apoptosis

Paraffin-embedded MDA-MB-231 xenografts were evaluated immunohistochemically for markers of tumor cell proliferation, survival, and apoptosis. Markers of proliferation and survival included Ki-67 (rabbit pAb; Lab Vision Corporation, Fremont, CA), phospho-specific p44/42 MAPK (Thr202/Tyr204) (rabbit pAb; Cell Signaling Technology) and phospho-specific Akt (Ser473) (rabbit pAb; Cell Signaling Technology). The EnVision+ System for rabbit antibodies (DAKO Cytomation, Carpenteria, CA) was used with 3,3' diaminobenzidine (DAB) as the chromagen, per kit instructions. After brief counterstaining in Mayer's hematoxylin all sections were dehydrated, cleared and coverslipped using a permanent mounting medium. Tumor apoptosis was assessed by TUNEL assay using ApopTag® Peroxidase In Situ Apoptosis Detection Kit (Chemicon, Temecula, CA) per kit instructions. Stained sections were coverslipped with Gelmount (Biomeda, Foster City, CA). Positive immunostaining and TUNEL positive immunofluorescence were analyzed and imaged using an Axioskop light microscope with an Axiocam digital camera (Carl Zeiss, Germany).

As shown in **Figure 27**, a marker for proliferating cells (Ki-67) was significantly reduced after 14 days of treatment with 18F1 at 20 mg/kg (about 0.5 mg/dose with female nu/nu athymic mice), 2x/week (Study Number 3067-04). In addition, 18F1 treatment resulted in a marked decrease in the activation of MAPK at this time point (**Figure 27**). An increase in apoptosis (**Figure 27**) as measured by TUNEL positive events and a significant decrease in Akt phosphorylation were also detected in MDA-MB-231 xenograft tumors after 1 week of treatment with 18F1 (0.5 mg/dose, M-W-F).

Example 11: *In Vivo* Blockage of Both Human and Murine VEGFR-1 Leads to Greater Antitumor Activity Against Human Breast Carcinoma Xenografts

18F1 was used in combination with an antibody to mouse VEGFR-1, MF1. 18F1 was diluted in 0.9% USP saline (Braun) or phosphate buffered saline (PBS) and administered intraperitoneally in a volume of 0.5 mL per mouse. The effect of treatment on tumor growth was analyzed using repeated measures analysis of variance (RM ANOVA), $p<0.05$ was considered significant As shown in **Figure 28**, in both the MDA-MB-231 (**Figure 28A**) and DU4475 (**Figure 28B**) xenograft models, inhibition of tumor expressed human VEGFR-1 with 18F1 and endogenous mouse VEGFR-1 with MF1, resulted in significant tumor growth inhibition ($p<0.05$). MF1 has previously been shown to inhibit tumor growth through a reduction in tumor angiogenesis. The combination of 18F1 and MF1 resulted in significantly more tumor growth inhibition than the monotherapies ($p<0.05$). 18F1 + MF1 combination therapy was not associated with body weight loss. These data support dual inhibition of tumor vascularization and tumor cell proliferation and survival with 18F1 treatment in patients.

Example 12: Anti-VEGFR-1 Antibody in Combination with Chemotherapeutics

18F1 + MF1 was combined with cytotoxic therapies, 5-flourouracil, leucovorin, and paclitaxel in the MDA-MB-231 model. 18F1 was diluted in 0.9% USP saline (Braun) or phosphate buffered saline (PBS). Antibody treatments administered at a constant dose per mouse were administered in a volume of 0.5 mL per mouse. Antibody and cytotoxic treatments administered at a dose proportional to body weight were given in a volume of 10 μL per gram body weight. 5-Fluorouracil

and leucovorin (5-FU/LV) were diluted separately in USP saline and dosed separately. Paclitaxel was either made in 5% benzyl alcohol (Sigma), 5% Cremophor EL (Sigma), and 90% USP saline or in 5% ethyl alcohol (Sigma), 5% Cremophor EL, and 90% USP saline. Cyclophosphamide and Doxorubicin were dissolved in USP saline for dosing. All treatments were administered i.p. The effect of treatments on tumor growth were analyzed using repeated measures analysis of variance (RM ANOVA), $p<0.05$ was considered significant.

As shown in **Figure 29**, in the MDA-MB-231 model, adding 18F1 + MF1 to an active dose of cyclophosphamide therapy significantly increased the antitumor effect. As shown in **Figure 30**, when 5-FU/LV and doxorubicin chemotherapy were administered at certain dose levels, 18F1 + MF1 increased the antitumor effects of these two chemotherapies.

In the DU4475 xenograft model, there was a trend for increased activity (lower T/C%) when IMC-18F1 + MF1 was combined with 5-FU/LV, doxorubicin and paclitaxel, although the effect did not reach statistical significance compared to the IMC-18F1 + MF1 alone, or cytotoxic agent monotherapy. In MDA-MB-231 this was again the case for doxorubicin, although for 5-FU/LV and paclitaxel there was no trend for increased activity with the combination. The lack of additivity may be due to the minimal effects of 5-FU/LV and paclitaxel as monotherapies at the selected dose levels. The combination with cyclophosphamide also had increased activity in the MDA-MB-435 model (T/C% = 51) compared to IMC-18F1 + MF1 alone (T/C% = 60) or cyclophosphamide monotherapy (T/C% =65), although these differences did not reach statistical significance. This was also the case for doxorubicin and paclitaxel in the same study. Similar to the MDA-MB-231 and MDA-MB-435 data above, a combination of IMC-18F1, MF1, and cyclophosphamide exhibited increased antitumor activity compared to antibody or cytotoxic therapy alone in a DU4475 xenograft model, although the trend did not reach statistical significance.

Statistical analysis

Tumor volumes and analysis of *in vitro* tumor cell growth were analyzed using Student's *t* test using the SigmaStat statistical package (v. 2.03; Jandel Scientific, San Rafael, CA). Differences of $p < 0.05$ were considered statistically significant.

Example 13: Cloning and sequencing of VH/VL regions of anti-VEGFR-1 antibodies

Poly (A+) mRNA was isolated from hybridoma cells producing clones 6F9, 13G12, 15F11, and 18F1 derived from VEGFR-1 immunized KM mice using a Fast-Track kit (Invitrogen, Carlsbad, CA). The generation of random primed cDNA was followed by polymerase chain reaction (PCR) using a Clontech kit. Primers (forward: 5'-ATGGAGTTTGGGCTGAGCTG and reverse: 3'-TGCCAGGGGGAAGACCGATGG) and (forward: 5'-ATG GAA ACC CCA GCG CAG CTT CTC and reverse: 3'-CGGGAAGATGAAGACAGATG) were used for binding to variable regions of heavy and kappa light chains, respectively. Sequences of human immunoglobulin-derived heavy and kappa chain transcripts from hybridomas were obtained by direct sequencing of PCR products generated from poly (A+) RNA using the primers described above. PCR products were also cloned into pCR2.1 using a TA cloning kit (Invitrogen, Carlsbad, CA) and both strands were sequenced using Prism dye-terminator sequencing kits and an ABI 3730 Sequencer (GENEWIZ, North Brunswick, NJ). All sequences were analyzed by alignments to the Kataman antibody sequence program using the DNASTAR software.

Table 2, above, shows amino acid sequences of the light and heavy chain variable regions of anti-VEGFR-1 antibody clones 6F9, 13G12, 15F11, and 18F1. The sequences of CDR1, CDR2, and CDR3 domains are indicated by underlining. Table 3, above, shows nucleotide sequences of the cDNA encoding the heavy and light chain variable regions of clones 6F9, 13G12, 15F11, and 18F1

Example 14: Engineering and expression of human IgG1 anti-VEGFR-1 antibodies

The DNA sequences encoding the heavy and light chain variable regions of the anti-VEGFR-1 antibody clones 6F9, 13G12, 15F11, and 18F1 were amplified by PCR for cloning into expression vectors. The heavy chain variable regions were fused in frame to the human immunoglobulin heavy chain gamma1 constant region in vector pEE6.1 (Lonza Biologics plc, Slough, Berkshire, UK). The entire human light chain cDNA was cloned directly into vector pEE12.1 (Lonza Biologics PLC, Slough, Berkshire, UK). Engineered immunoglobulin expression vectors were stably transfected in NS0 myeloma cells by electroporation and selected in glutamine synthetase selection medium. Stable clones were screened for antibody expression by anti-Fc and VEGFR-1 specific binding ELISA. Positive clones were expanded into

serum-free medium culture for antibody production in spinner flasks or bioreactors for a period of up to two weeks. Full length IgG1 antibody was purified by protein A affinity chromatography (Poros A, PerSeptive Biosystems Inc., Foster City, CA) and eluted into a neutral buffered saline solution.

The foregoing description and examples have been set forth merely to illustrate the invention and are not intended as being limiting. Each of the disclosed aspects and embodiments of the present invention may be considered individually or in combination with other aspects, embodiments, and variations of the invention. In addition, unless otherwise specified, none of the steps of the methods of the present invention are confined to any particular order of performance. Modifications of the disclosed embodiments incorporating the spirit and substance of the invention may occur to persons skilled in the art and such modifications are within the scope of the present invention. Furthermore, all references cited herein are incorporated by reference in their entirety.

What is claimed is:

1. An isolated human monoclonal antibody or fragment thereof that binds specifically to VEGFR-1 comprising a light chain complementarity determining region-2 (CDR2) of SEQ ID NO:2 and a light chain CDR3 of SEQ ID NO:3.

2. The antibody or fragment thereof of claim 1, further comprising a light chain CDR1 region having the following sequence:

$$RASQSX_1SSSYLA,$$

wherein X_1 is V or G.

3. The antibody or fragment thereof of claim 1, further comprising a heavy chain CDR1 having the following sequence:

$$GFX_2FSSYGMH,$$

wherein X_2 is T or A.

4. The antibody or fragment thereof of claim 1, further comprising a heavy chain CDR2 having the following sequence:

$$VIWX_3DGSNKYYADSVX_4G,$$

wherein X_3 is Y or F and X_4 is K or R.

5. The antibody or fragment thereof of claim 1, further comprising a heavy chain CDR3 having the following sequence:

$$DHX_5GSGX_6HX_7YX_8YYGX_9DV$$

wherein X_5 is F or Y; X_6 is A or V; X_7 is Y, S, or H; X_8 is Y or F; and X_9 is M or L.

6. An antibody or fragment thereof comprising (i) a light chain variable region selected from the group consisting of SEQ ID NO:14, 15, and 16 or (ii) a heavy chain variable region selected from the group consisting of SEQ ID NO:17, 18, 19, and 20.

7. An isolated polynucleotide comprising a nucleotide sequence encoding the antibody or fragment thereof of claim 1.

8. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, the nucleotide sequence encoding an antibody or fragment thereof that specifically binds to VEGFR-1.

9. An expression vector comprising the polynucleotide sequence of claim 7 linked to an expression sequence.

10. A recombinant host cell comprising the expression vector of claim 9.

11. The recombinant host cell of claim 10, or a progeny thereof, wherein the cell expresses the antibody or fragment thereof of claim 7 .

12. A method of producing an antibody or fragment thereof comprising culturing the cell of claim 10 under conditions permitting expression of the antibody or fragment thereof of claim 7.

13. A method of modulating activity of VEGFR-1 in a mammal comprising administering to the mammal an effective amount of an antibody or fragment thereof of claim 1.

14. A method of inhibiting angiogenesis in a mammal comprising administering to the mammal an effective amount of an antibody or fragment thereof of claim 1.

15. A method of reducing tumor growth in a mammal comprising administering to the mammal an effective amount of an antibody or fragment thereof of claim 1.

16. The method of claim 15, wherein the method further comprises administering an anti-neoplastic agent or treatment.

17. The method of claim 15, wherein the tumor is a breast tumor.

Figure 1. Amino acid sequence of the human anti-VEGFR-1 antibodies

6F9-Light chain

METPAQLLFLLLLWLPESTGEIVLTQSPGTLSLSPGERATLSCRASQSGSSSYLAWYQQKPGQAPRLLI
Y

CDR1

GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIKRTVAAPSVFIFP
CDR2 CDR3

6F9-Heavy chain

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW
CDR1

VAVIWYDGSNKYYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYHCTRDHFGSGAHYYYYYGMD
V

CDR2 CDR3

WGQGTTVTVSS

13G12-Light chain

METPAQLLFLLLLWLPESTGEIVLTQSPGTLSLSPGERATLSCRASQSGSSSYLAWYQQKPGQAPRLLI
Y

CDR1

GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIKRTVAAPSVFIFP
CDR2 CDR3

13G12-Heavy chain

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW
CDR1

VAVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDHYGSGAHYYYYYGMD
V

CDR2 CDR3

WGQGTTVTVSS

15F11-Light chain

MEAPAQLLFLLLLWLPDTTGEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY
CDR1

GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGQGTRLEIKRTVAAPSVFIFP
CDR2 CDR3

15F11-Heavy chain

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW
CDR1

VAVIWFDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDHYGSGAHSYYYYGLDV
CDR2 CDR3

WGQGTSVTVSS

18F1-Light chain

METPAQLLFLLLLWLPDTTGEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY
CDR1

GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIKRTVAAPSVFIFP
CDR2 CDR3

18F1-Heavy chain

MEFGLSWVFLVALLRGVQCQAQVVESGGGVVQSGRSLRLSCAASGFAFSSYGMHWVRQAPGKGLE
W

CDR1

VAVIWYDGSNKYYADSVRGRFTISRDNSENTLYLQMNSLRAEDTAVYYCARDHYGSGVHHYFYYGLDV
CDR2 CDR3

WGQGTTVTVSS

Figure 2. Nucleotide sequence of the human anti-VEGFR-1 antibodies

6F9-Light chain

ATGGAAACCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGAAAGCACCGGAGAAATTGTGTTGACGC
AGTCTCCAGGCACCCTGTCCTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGGTAGCAG
CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCC
ACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTG
AAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGAT
CAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCG

6F9-Heavy chain

ATGGAGTTTGGGCTGAGCTGGGTTTTCCTCGTTGCTCTTTTAAGAGGTGTCCAGTGTCAGGTGCAGCTGGTGGAGT
CTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAGTTA
TGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAAT
AAATACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGGTGTATCTGCAAA
TGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATCACTGTACGAGAGATCACTTTGGTTCGGGGGCTCACTACTA
CTACTACTACGGTATGGACGTCTGGGGCCAAgGGACCACGGTCACCGTCTCCTCA

13G12-Light chain

ATGGAAACCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGAAAGCACCGGAGAAATTGTGTTGACGC
AGTCTCCAGGCACCCTGTCCTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGGTAGCAG
CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCC
ACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTG
AAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGAT
CAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCG

13G12-Heavy chain

ATGGAGTTTGGGCTGAGCTGGGTTTTCCTCGTTGCTCTTTTAAGAGGTGTCCAGTGTCAGGTGCAGCTGGTGGAGT
CTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTA
TGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAAT
AAATACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAA
TGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCACTATGGTTCGGGGGCTCACTACTA
CTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACgGTCACCGTCTCCTCA

15F11-Light chain

ATGGAAGCCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGATACCACCGGAGAAATTGTGTTGACGC
AGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG
CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCC
ACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTG
AAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCTCTCACCTTCGGCCAAGGGACACGACTGGAGAT
TAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCG

15F11-Heavy chain

ATGGAGTTTGGGCTGAGCTGGGTTTTCCTCGTTGCTCTTTTAAGAGGTGTCCAGTGTCAGGTGCAGCTGGTGGAGT
CTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTA
TGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTTTGATGGAAGTAAT
AAATACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAA
TGAACAGCCTGAGAgCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCACTATGGTTCGGGGGCTCACTCCTA
CTACTACTACGGTTTGGACGTTTGGGGCCAAGGGACCTCGGTCACCGTCTCCTCA

18F1-Light chain

ATGGAAACCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGATACCACCGGAGAAATTGTGTTGACGC
AGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAG
CAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCC
ACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTG
AAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGAT
CAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTTCCG

18F1-Heavy chain

ATGGAGTTTGGGCTGAGCTGGGTTTTCCTCGTTGCTCTTTTAAGAGGTGTCCAGTGTCAGGCGCAGGTGGTGGAGT
CTGGGGGAGGCGTGGTCCAGTCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCGCCTTCAGTAGCTA
CGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAAT
AAATACTATGCAGACTCCGTGAGGGGCCGATTCACCATCTCCAGAGACAATTCCGAGAACACGCTGTATCTGCAAA
TGAACAGCCTGAGAGCCGAGGACACCGCTGTATATTACTGTGCCAGAGATCACTATGGTTCGGGGGTGCACCACTA
TTTCTACTACGGTCTGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

Figure 3

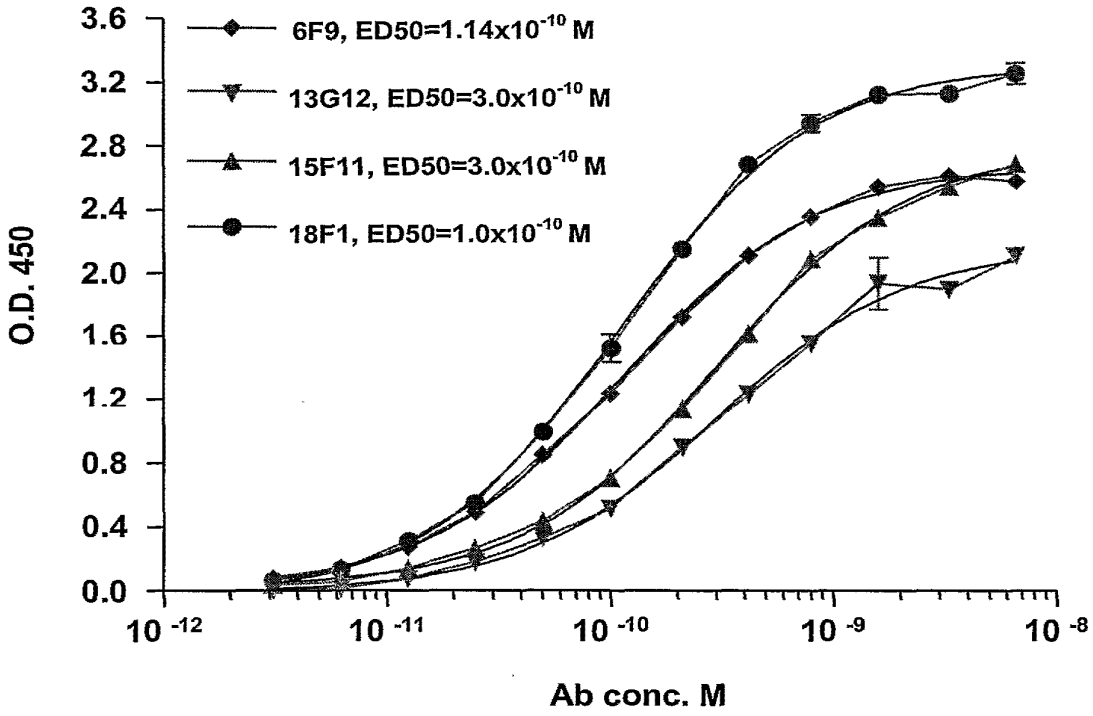

6F9, ED50=1.14x10-10 M
13G12, ED50=3.0x10-10 M
15F11, ED50=3.0x10-10 M
18F1, ED50=1.0x10-10 M
O.D. 450
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
10 -12
10 -11
10 -10
10 -9
10 -8
Ab conc. M

Figure 4

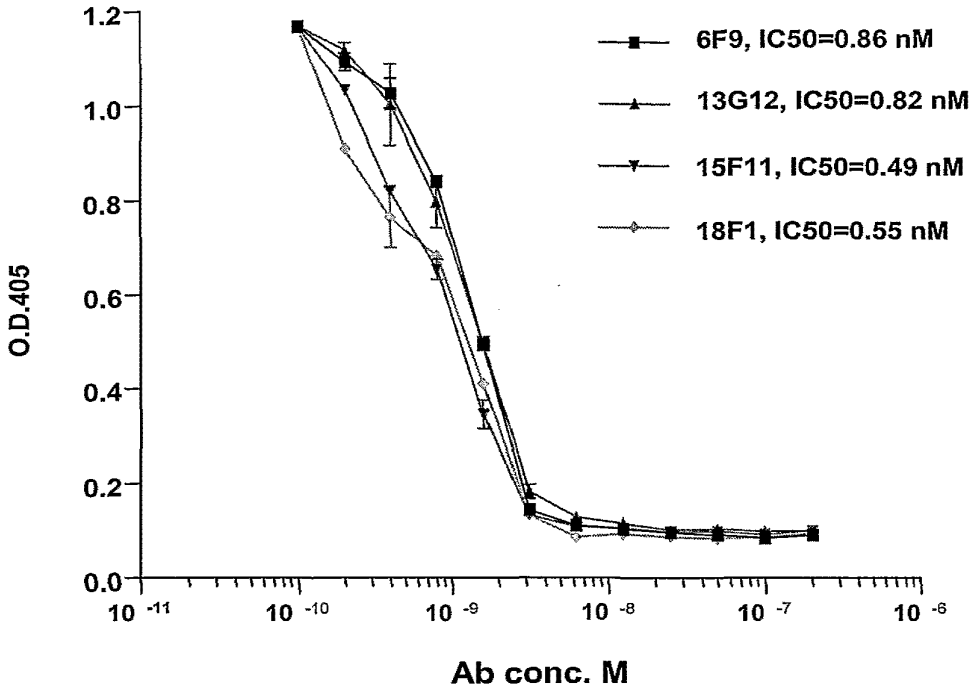

1.2
1.0
0.8
0.6
0.4
0.2
0.0
O.D.405
10 -11
10 -10
10 -9
10 -8
10 -7
10 -6
Ab conc. M
6F9, IC50=0.86 nM
13G12, IC50=0.82 nM
15F11, IC50=0.49 nM
18F1, IC50=0.55 nM

Figure 5

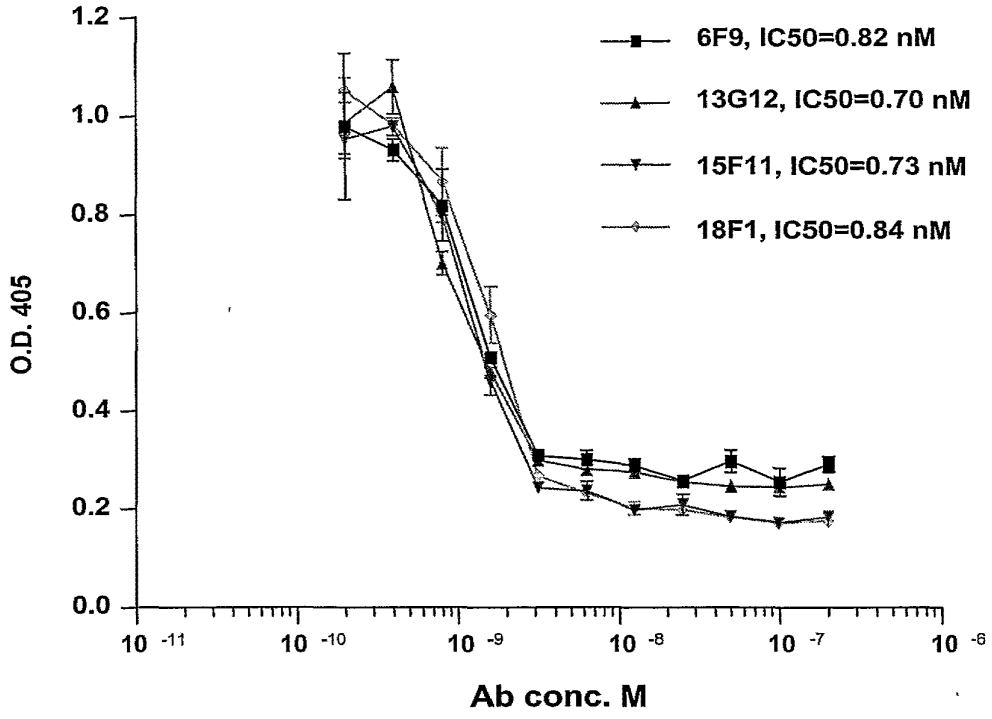

1.2
1.0
0.8
0.6
0.4
0.2
0.0
O.D. 405
10 -11
10 -10
10 -9
10 -8
10 -7
10 -6
Ab conc. M
6F9, IC50=0.82 nM
13G12, IC50=0.70 nM
15F11, IC50=0.73 nM
18F1, IC50=0.84 nM

Figure 6

Figure 6A

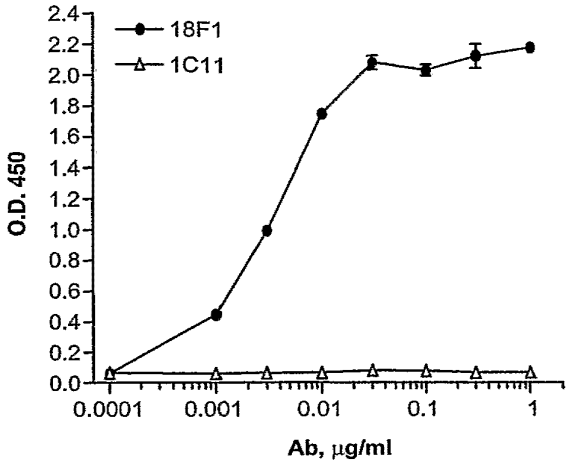
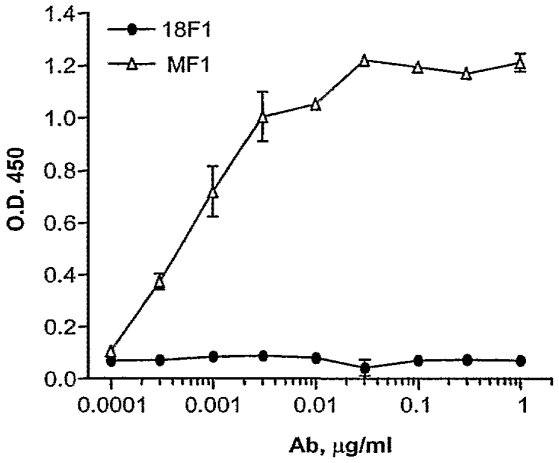


Figure 6B



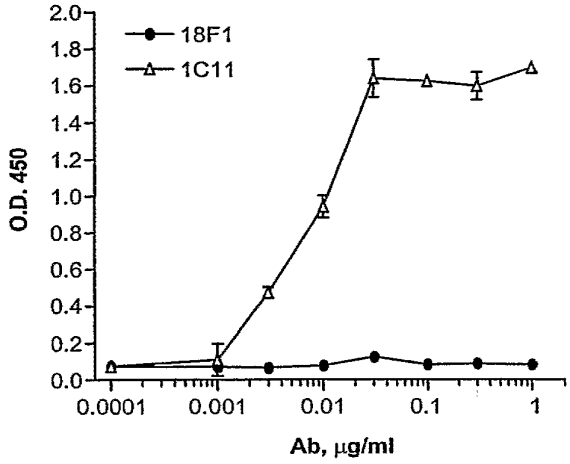


Figure 6C

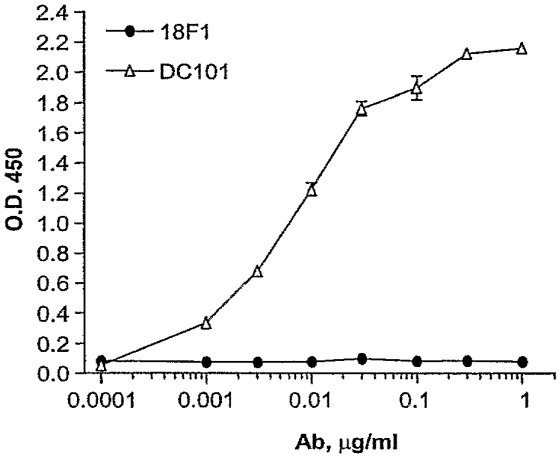


Figure 6D

Figure 7

Figure 7A

Figure 7B

Figure 7C

Figure 7D

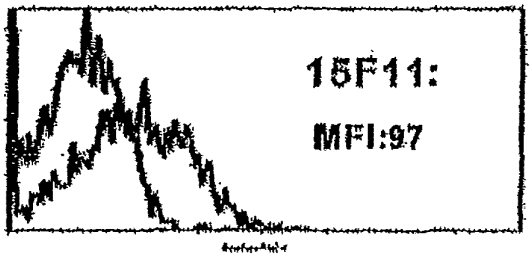


Figure 7E

Figure 8

Figure 8A

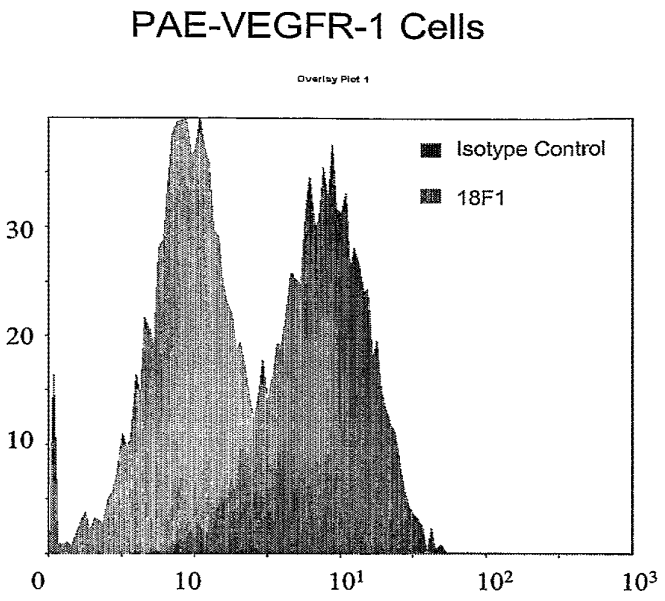


Figure 8B

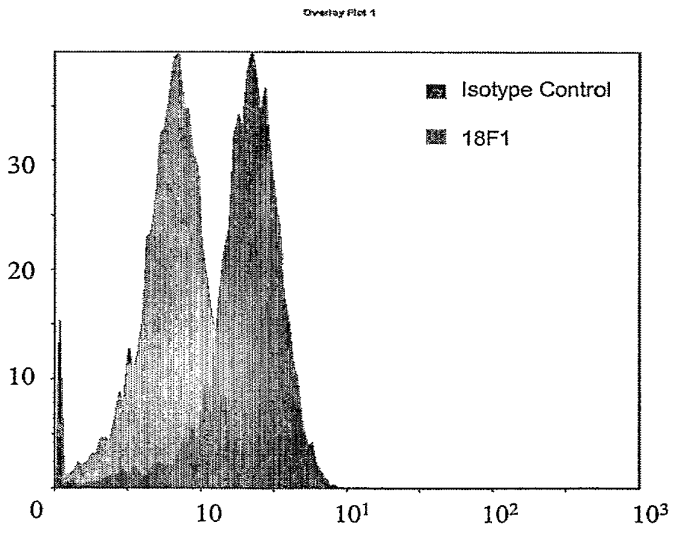


Figure 9

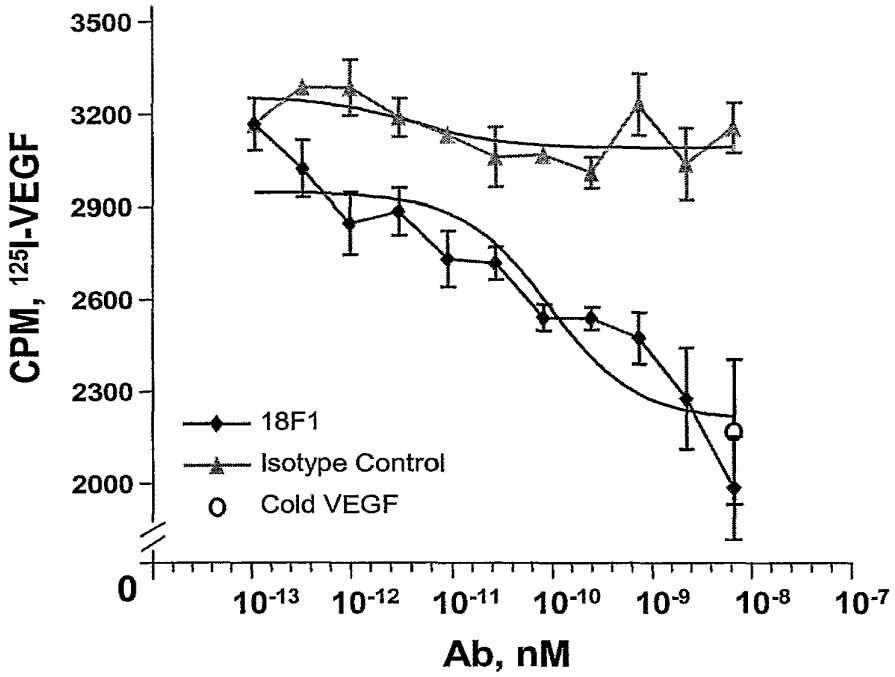

3500
3200
2900
2600
2300
2000
0
CPM, 125I-VEGF
18F1
Isotype Control
Cold VEGF
10-13
10-12
10-11
10-10
10-9
10-8
10-7
Ab, nM

Figure 10

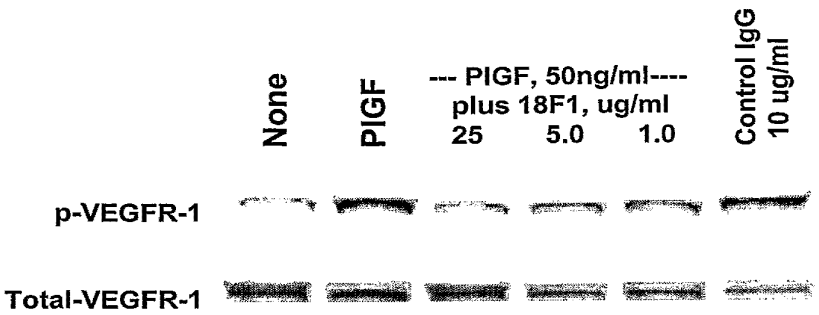

None
PIGF
--- PIGF, 50ng/ml----
plus 18F1, ug/ml
25
5.0
1.0
Control IgG
10 ug/ml
p-VEGFR-1
Total-VEGFR-1

Figure 11

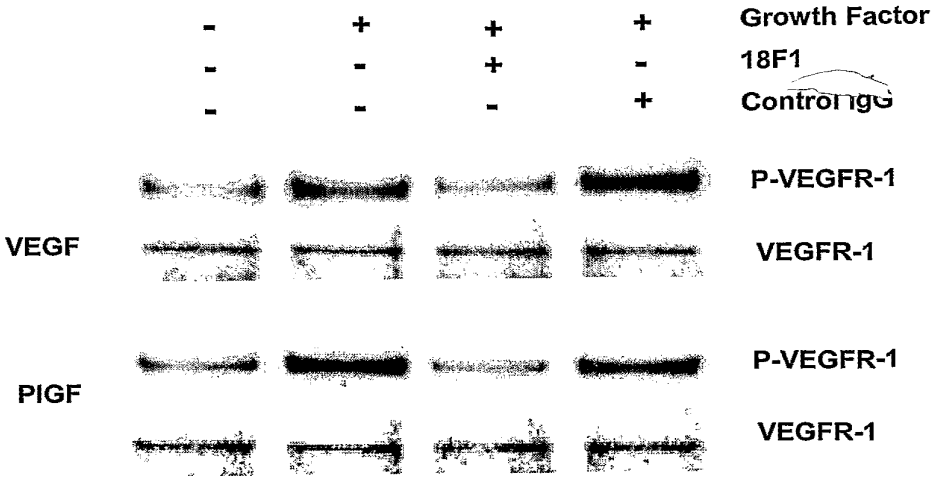

- + + + Growth Factor
- - + - 18F1
- - - + Control IgG
P-VEGFR-1
VEGF
VEGFR-1
P-VEGFR-1
PIGF
VEGFR-1

Figure 12

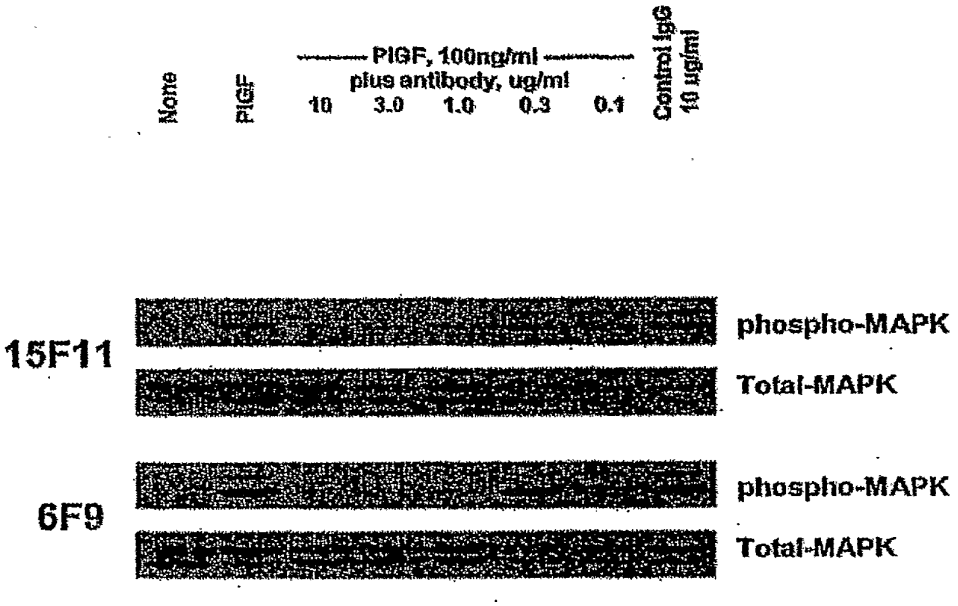

None
PlGF
PlGF, 100ng/ml
plus antibody, ug/ml
10
3.0
1.0
0.3
0.1
Control IgG
10 ug/ml
15F11
phospho-MAPK
Total-MAPK
6F9
phospho-MAPK
Total-MAPK

Figure 13

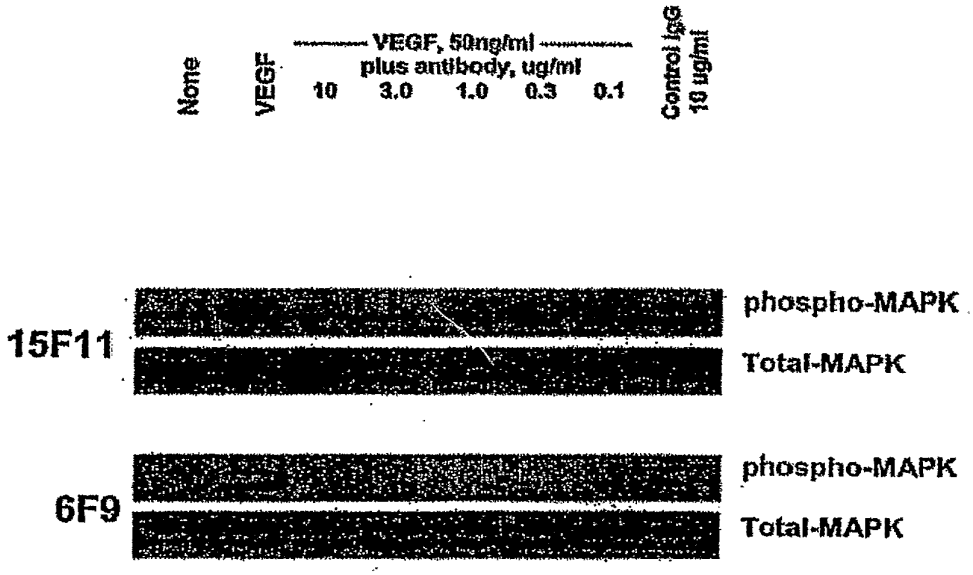

None
VEGF
VEGF, 50ng/ml
plus antibody, ug/ml
10
3.0
1.0
0.3
0.1
Control IgG
10 ug/ml
15F11
phospho-MAPK
Total-MAPK
6F9
phospho-MAPK
Total-MAPK

Figure 14

Figure 14A

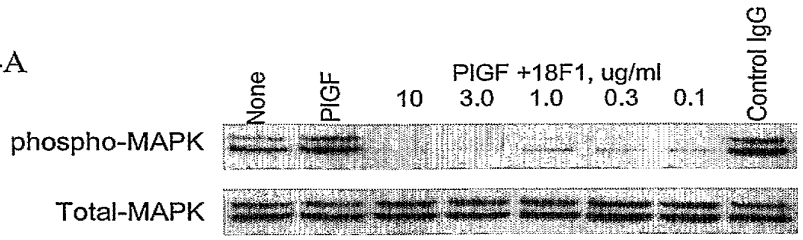


Figure 14B

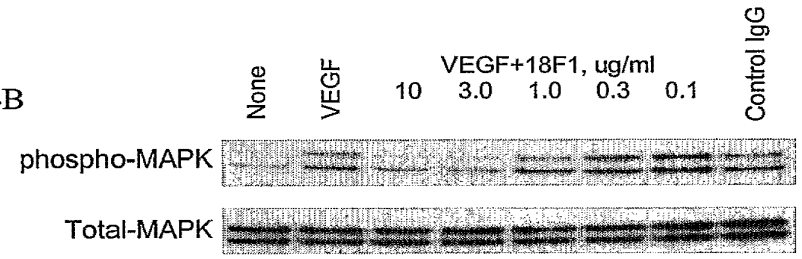


Figure 15

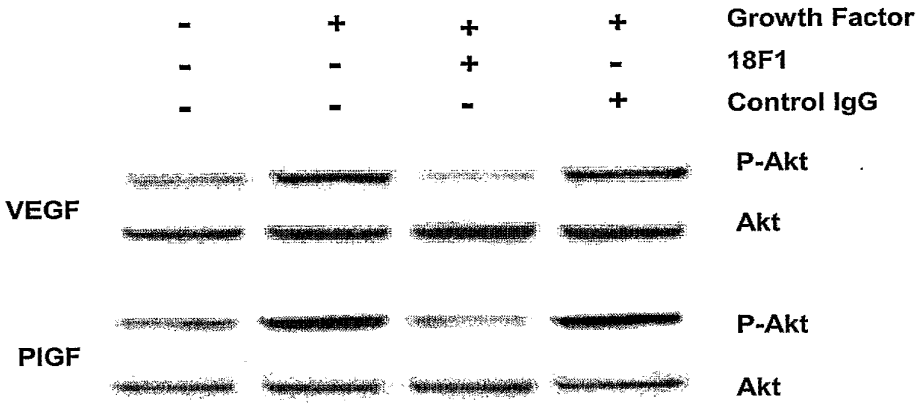


Figure 16

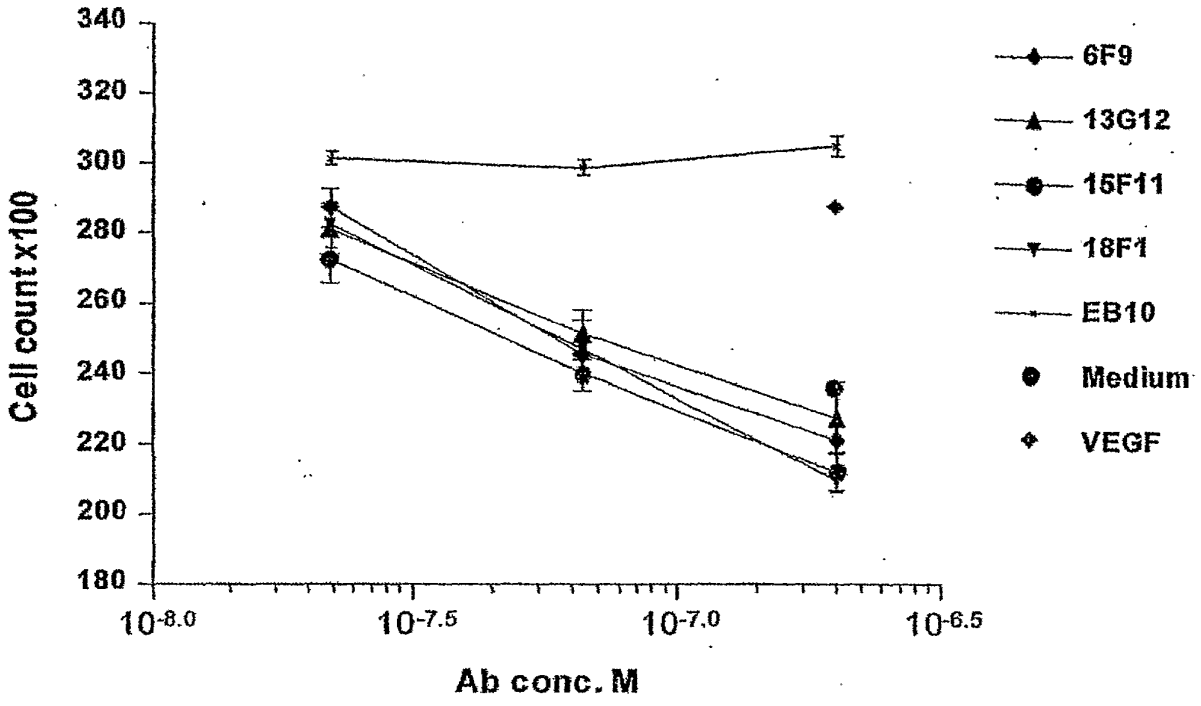

340
320
300
280
260
240
220
200
180
Cell count x100
10-8.0
10-7.5
10-7.0
10-6.5
Ab conc. M
6F9
13G12
15F11
18F1
EB10
Medium
VEGF

Figure 17

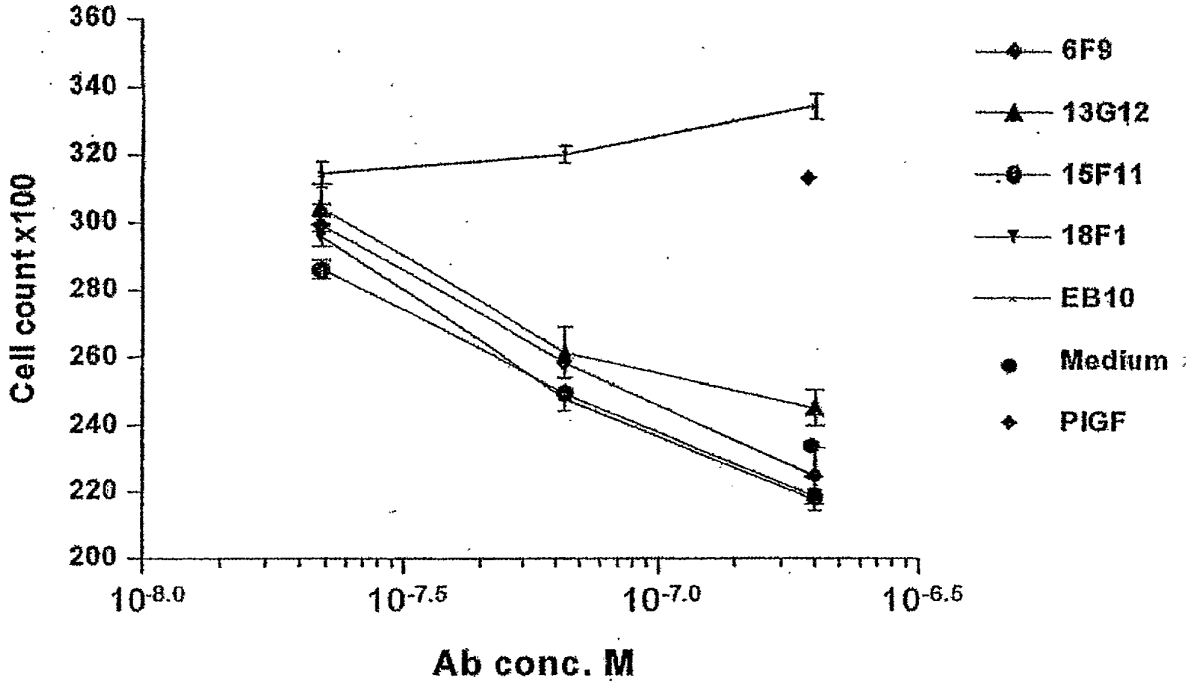
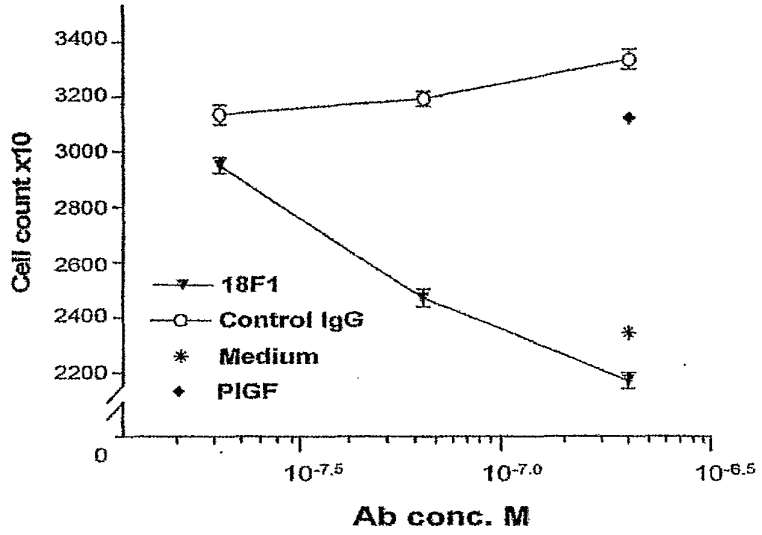

360
340
320
300
280
260
240
220
200
Cell count x100
10-8.0
10-7.5
10-7.0
10-6.5
Ab conc. M
6F9
13G12
15F11
18F1
EB10
Medium
PlGF

Figure 18


3400
3200
3000
2800
2600
2400
2200
0
Cell count x10
18F1
Control IgG
Medium
PIGF
10-7.5
10-7.0
10-6.5
Ab conc. M

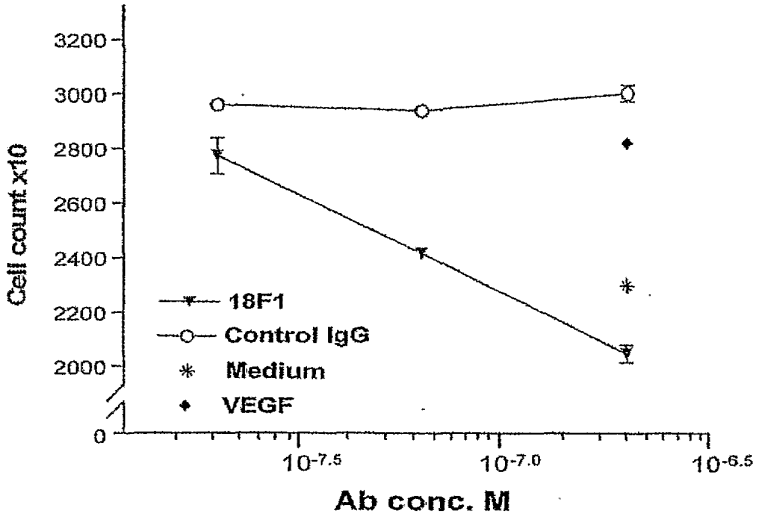
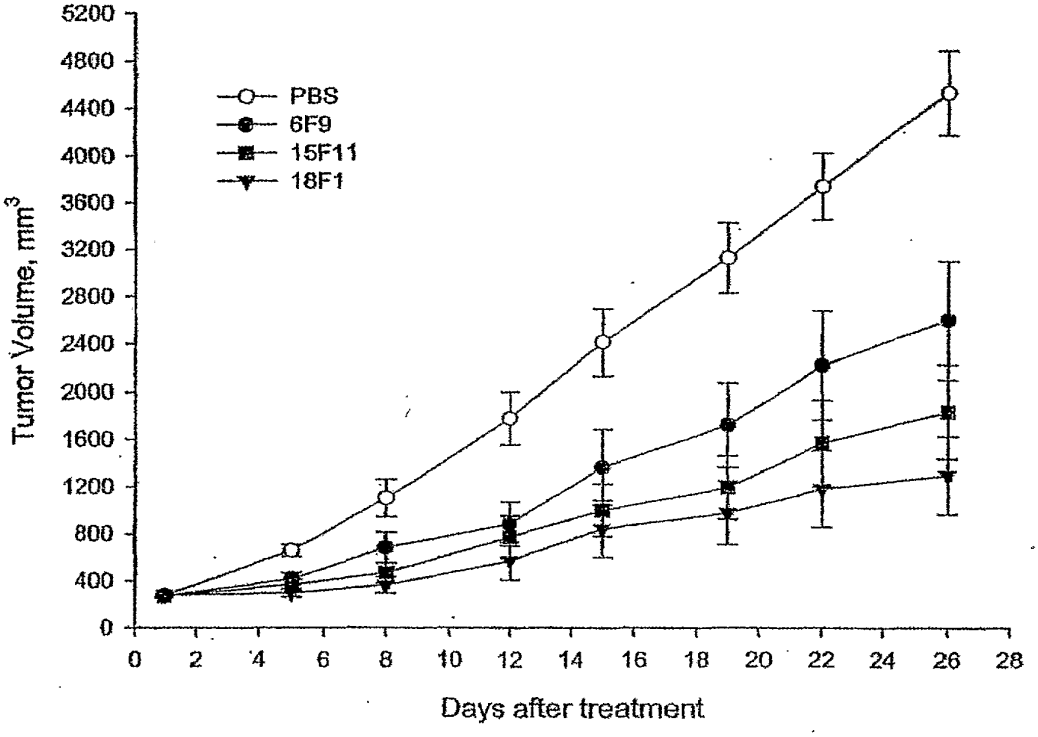

3200
3000
2800
2600
2400
2200
2000
0
Cell count x10
18F1
Control IgG
Medium
VEGF
10-7.5
10-7.0
10-6.5
Ab conc. M

Figure 19A


PBS
6F9
15F11
18F1
Tumor Volume, mm3
Days after treatment
0
400
800
1200
1600
2000
2400
2800
3200
3600
4000
4400
4800
5200
0
2
4
6
8
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28

Figure 19B

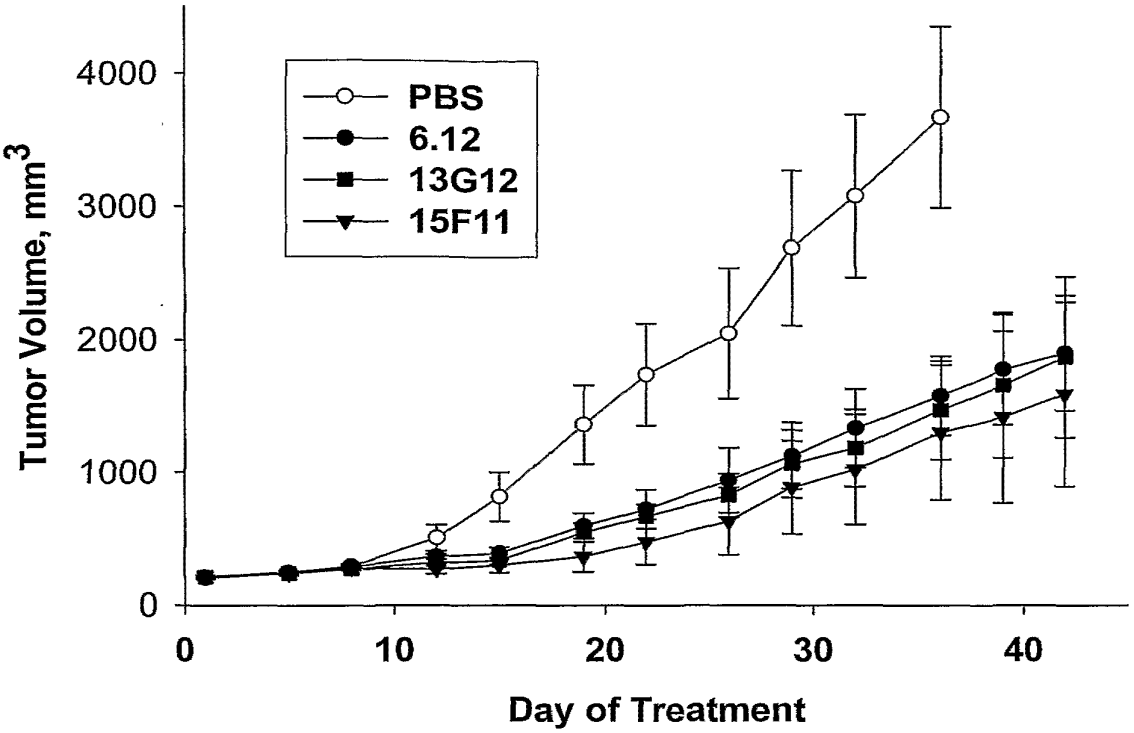

PBS
6.12
13G12
15F11
Tumor Volume, mm3
4000
3000
2000
1000
0
0
10
20
30
40
Day of Treatment

Figure 20

Figure 20A

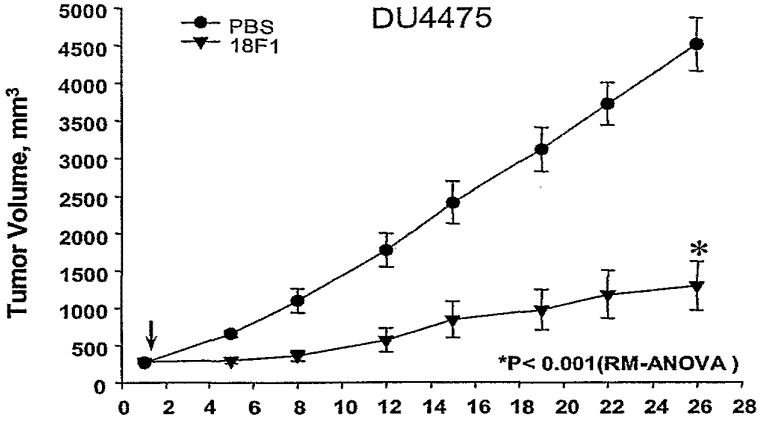


Figure 20B

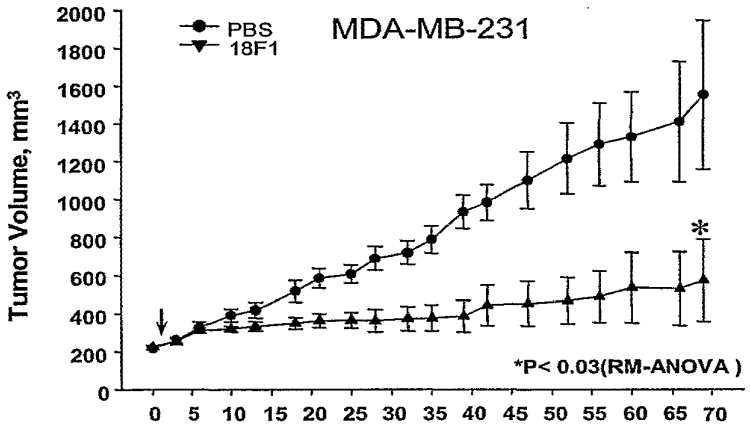


Figure 20C

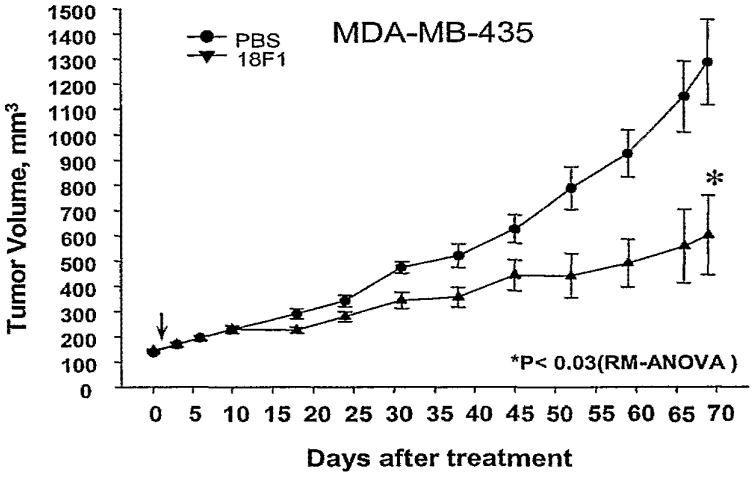


Figure 21

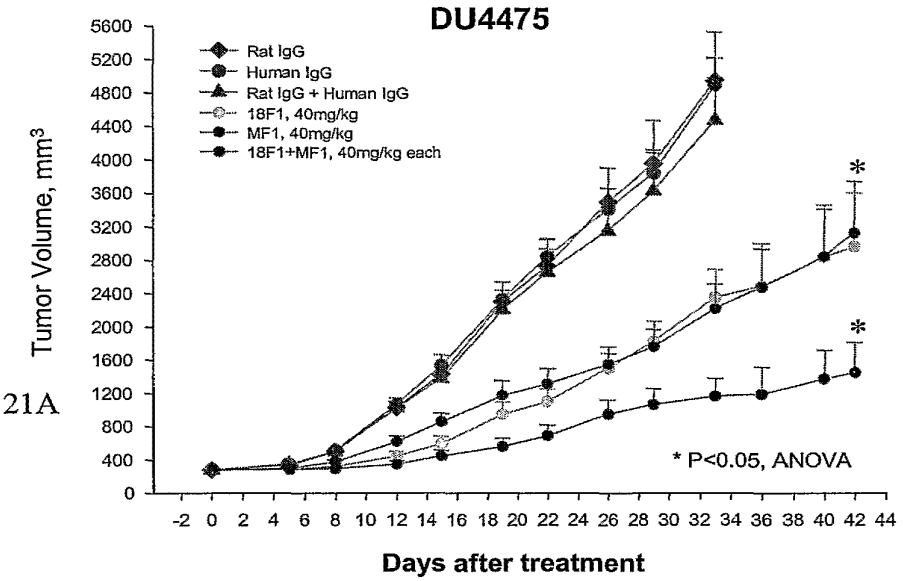


Figure 21A

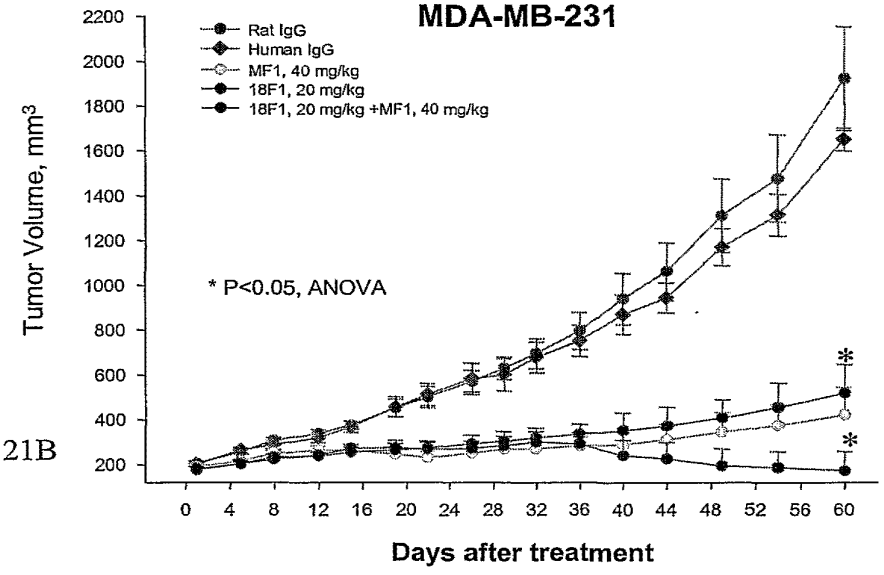


Figure 21B

Figure 22

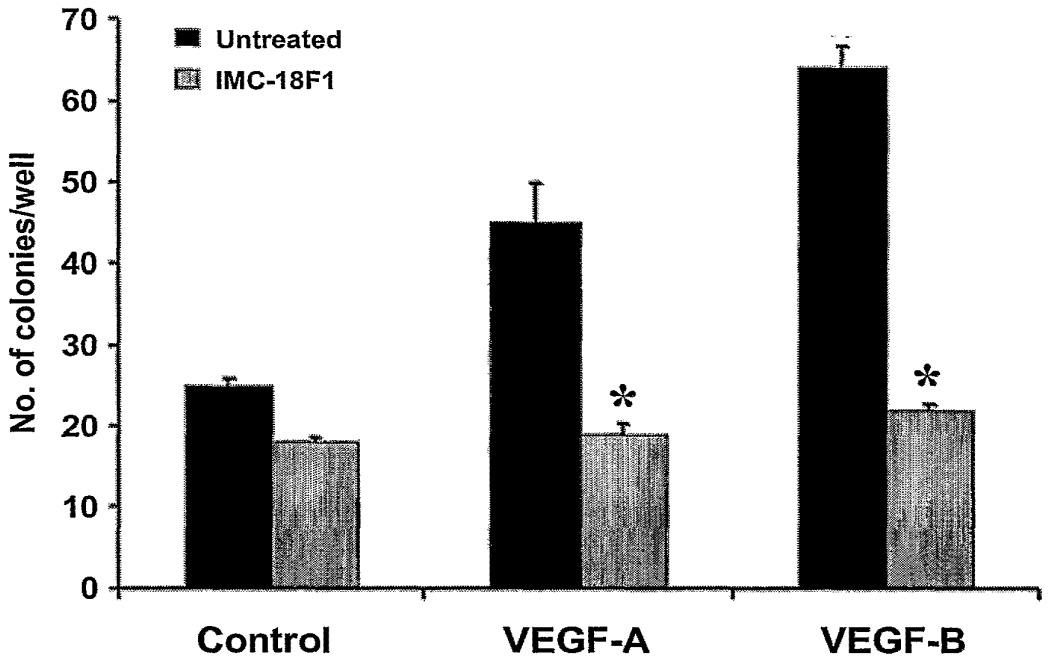


Figure 22: Inhibition of Tumor Cell Colony Formation. HT29 tumor cells were seeded in medium containing soft agar with or without 18F1 in the presence of VEGF-A or VEGF-B and incubated for 14 days. Colonies were counted under microscope. Treatment with 18F1 decreased colony formation induced by the VEGF ligands ($*P < 0.03$). Bars indicate SEM. Data from Pavco *et al*; Clin. Cancer Res. 2000; 6:2094-103.

Figure 23

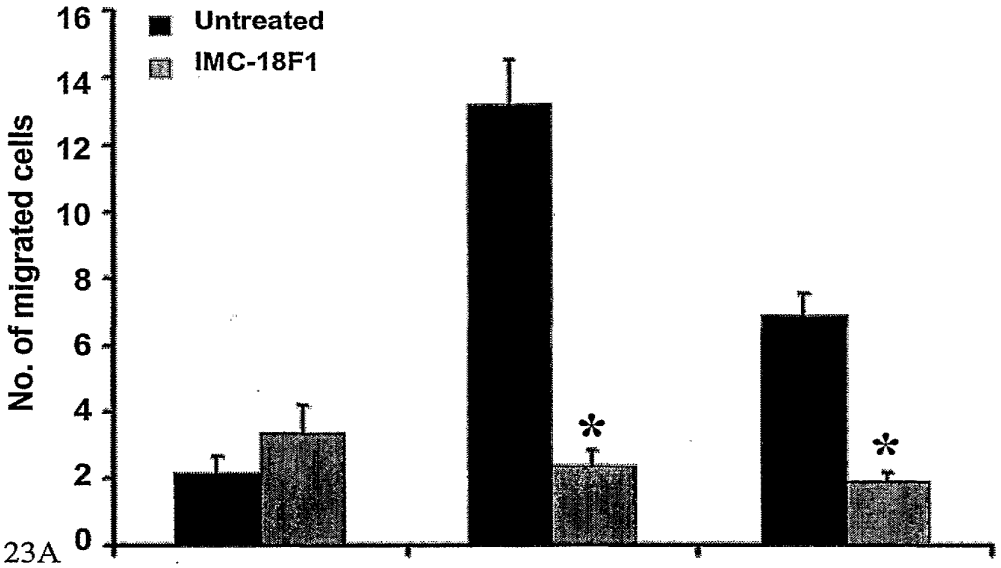


Figure 23A

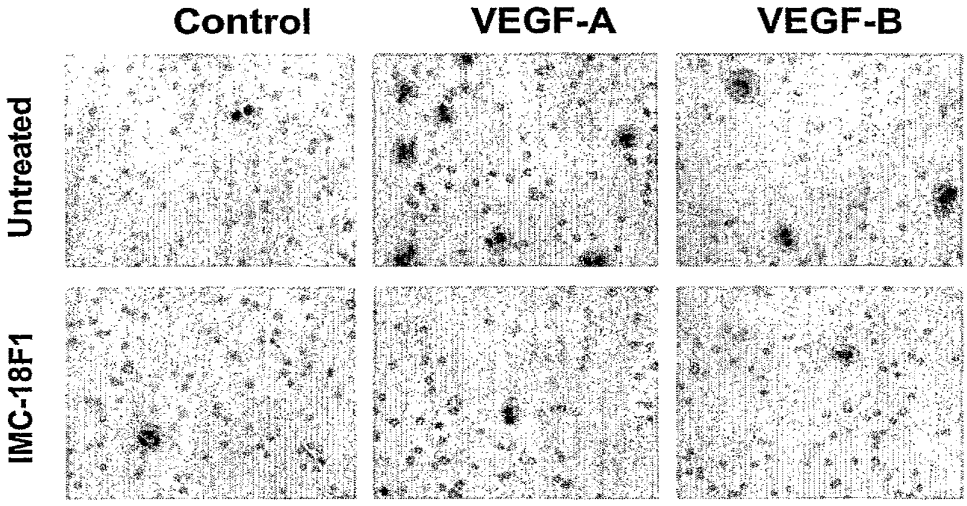


Figure 23B

Figure 23A and **B**: Inhibition of Tumor Cell Migration. (A) HT-29 tumor cells were treated with 18F1 in the presence of VEGF-A or VEGF-B for 48 hours. Cell migration across a layer of Matrigel is plotted as Mean $\pm$ SEM (*$P < 0.0001$). (B) Photomicrographs of migrated cells stained with Diff-Quik. Data from Pavco *et al*; Clin. Cancer Res. 2000; 6:2094-103.

Figure 24

Figure 24A

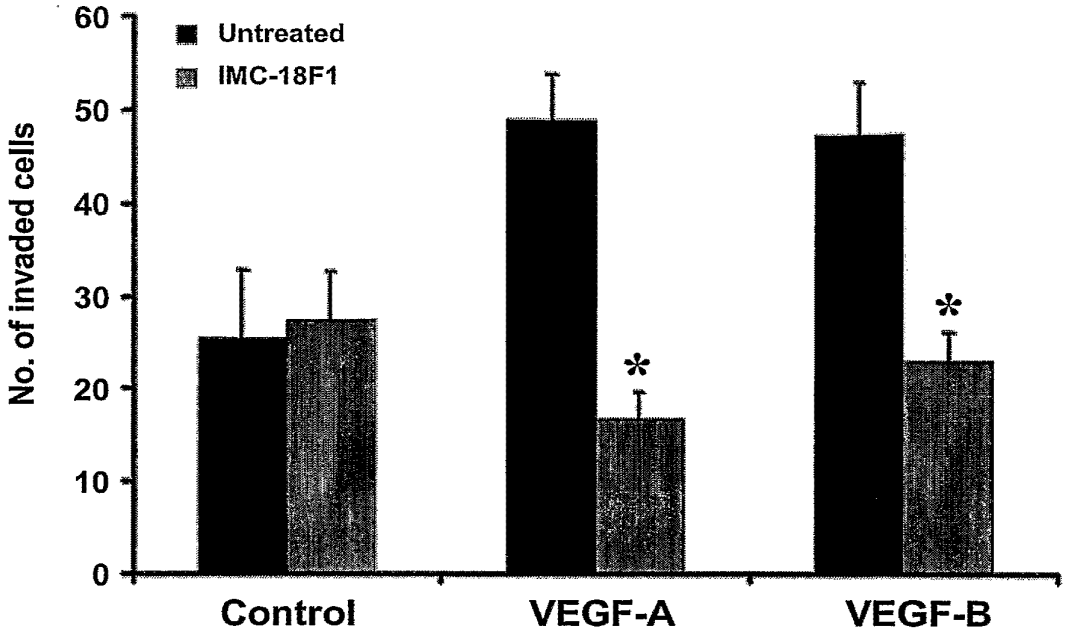


Figure 24B

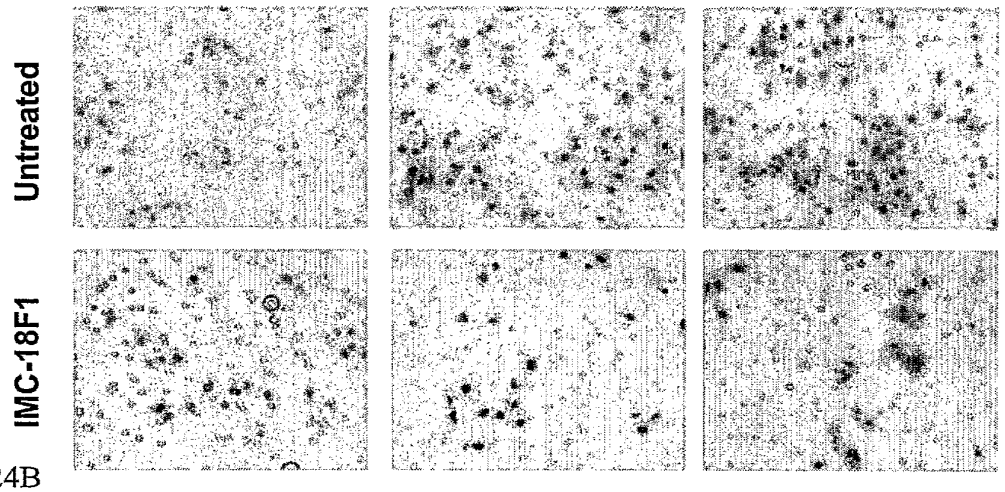
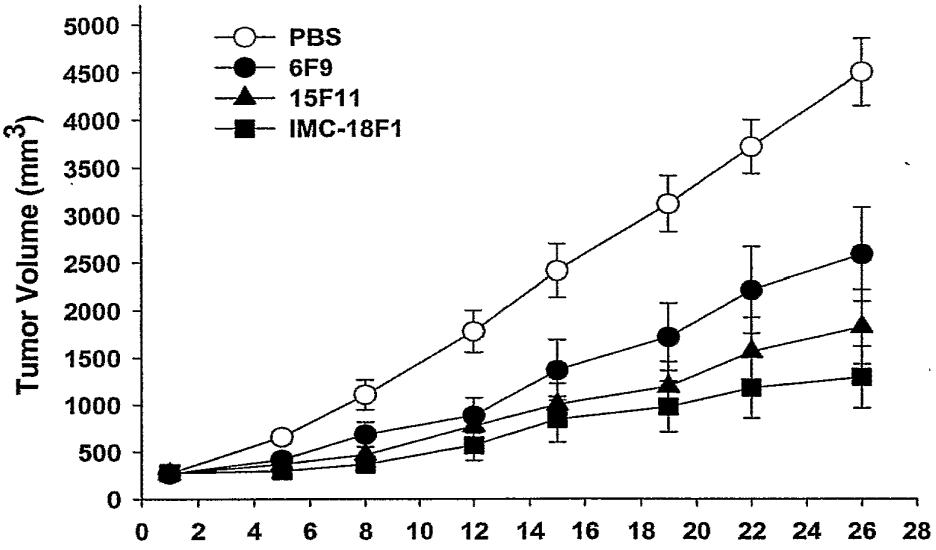


Figure 24A and **B:** Inhibition of Tumor Cell Invasion. (A) SW480 tumor cells were treated with 18F1 in the presence of VEGF-A or VEGF-B for 48 hours. Cell migration across a layer of Matrigel is plotted as Mean ± SEM (*$P < 0.0001$). (B) Photomicrographs of migrated cells stained with Diff-Quik. Data from Pavco *et al*; Clin. Cancer Res. 2000; 6:2094-103.

Figure 25

Figure 25A



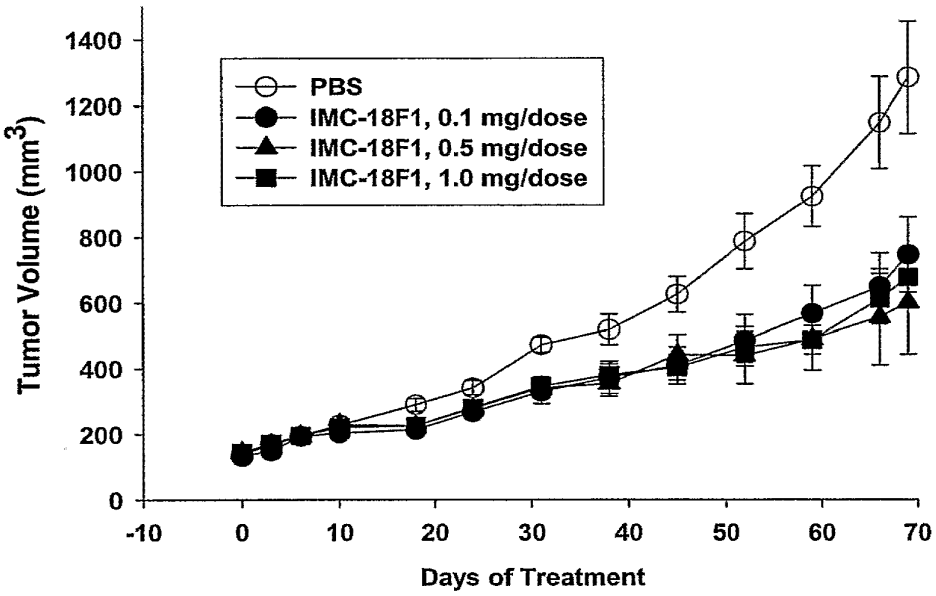


Figure 25B

Figure 25A and **B**: Effect of 18F1 Monotherapy on Breast Cancer Xenografts. (A) Mice with DU4475 xenografts were treated with 18F1, 6F9, or 15F11 antibodies to VEGFR-1, at 1.0 mg/dose, M-W-F. (B) Mice with MDA-MB-435 xenografts were treated with 18F1 at the indicated dosages or PBS at 0.5 mL/dose, M-W-F. Mean tumor volume ± SEM is plotted for n=12 per group.

Figure 26

Figure 26A

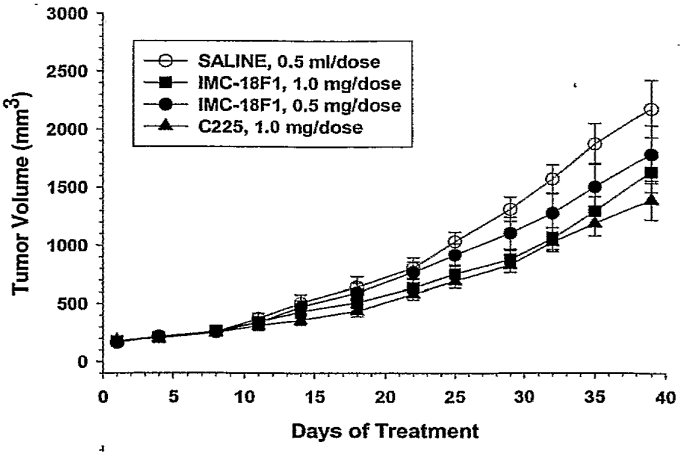


Figure 26B

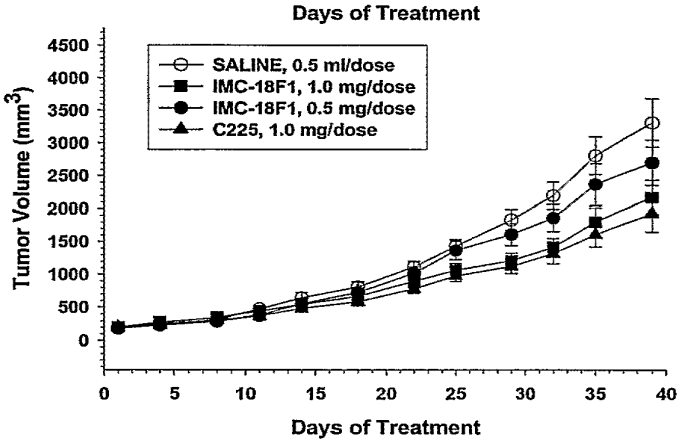


Figure 26C

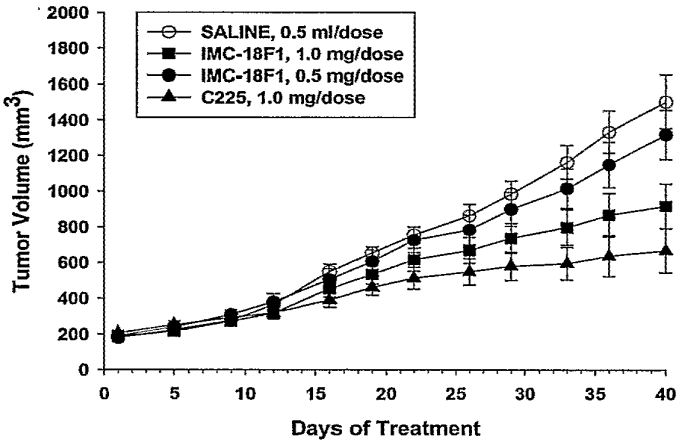


Figure 26A, B, and **C**: Effect of 18F1 Monotherapy on Colon Cancer Xenografts. Mice with HT-29 (A), DLD-1 (B), and GEO (C) xenografts were treated with 18F1 at the indicated dosages, M-W-F. Mean tumor volume ± SEM is plotted for n=10 per group.

Figure 27

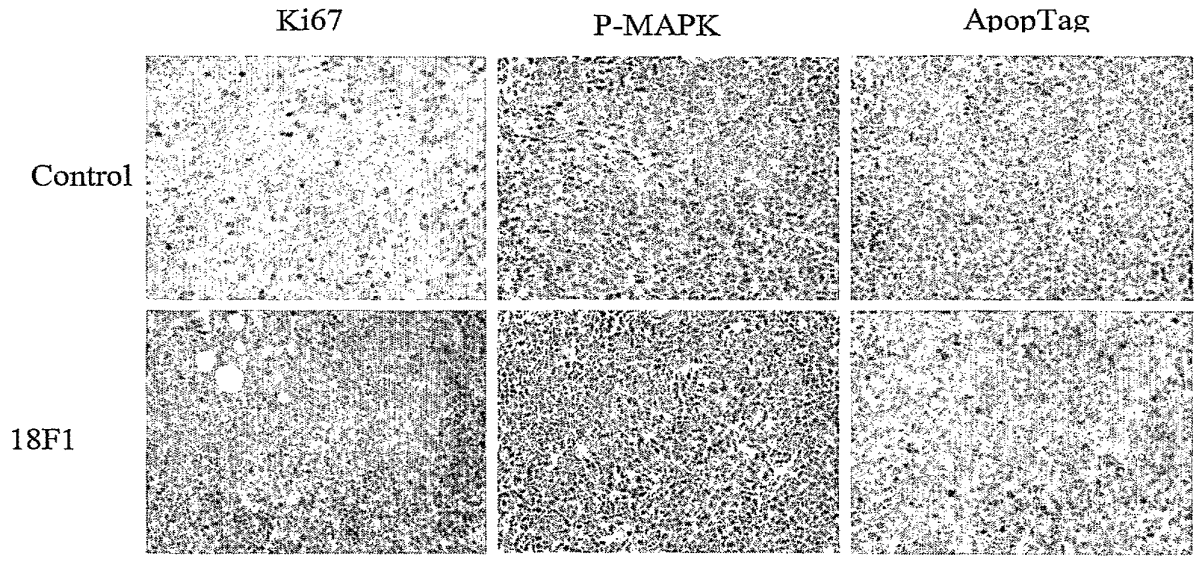


Figure 27: Ki-67, phosphorylated MAPK (P-MAPK) and TUNEL (ApopTag) Staining in MDA-MB-231 Xenograft Tumors. Tumor sections were stained for Ki67, P-MAPK (14 days of 18F1 treatment at 20 mg/kg, 2x/week); or ApopTag (7 days of 18F1 treatment at 0.5 mg/dose, 3x/week) Representative tumor sections are shown. Control indicates the matching dose of Human IgG.

Figure 28

Figure 28A

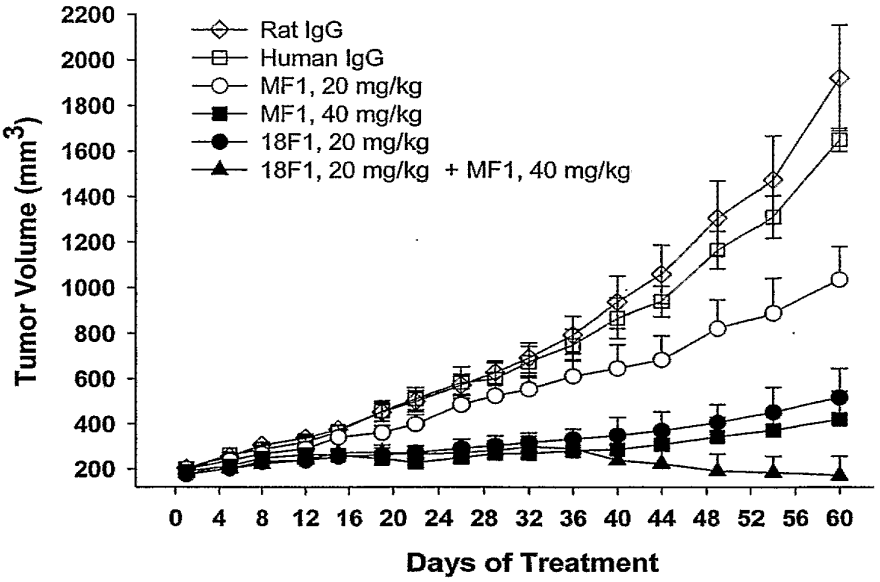


Figure 28B

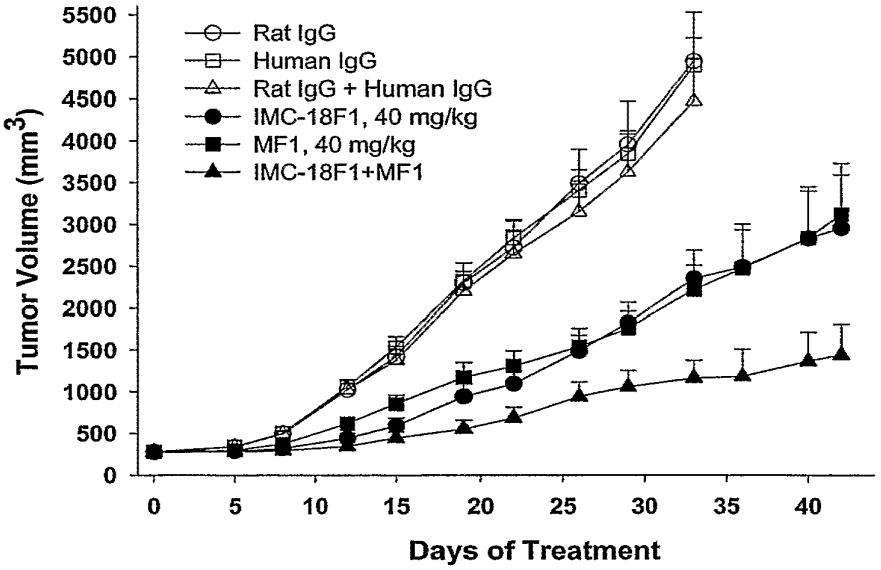


Figure 28A and **B**: Effect of Combined Inhibition of Mouse and Human VEGFR-1 on Xenograft Growth. Mice with MDA-MB-231 (A) or DU4475 (B) xenografts were treated with 18F1, MF1 or the combination, at the indicated dosages (2x/week for 18F1 and 3x/week for MF1). Mean tumor volume ± SEM is plotted for n=16-18 per group.

Figure 29

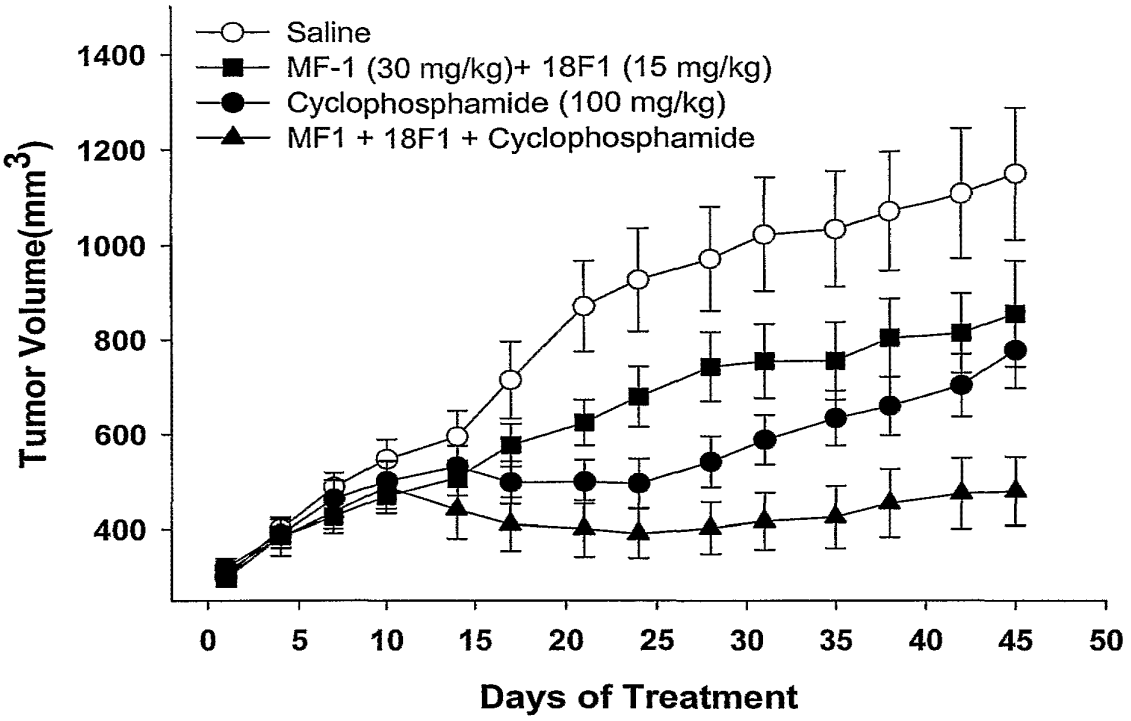


Figure 29: Effect of Cyclophosphamide Treatment in Combination with Anti-mouse and human VEGFR-1 Antibodies on the Growth of MDA-MB-231 Xenografts. Mice with MDA-MB-231 xenografts were treated with 18F1 + MF1 alone (2x/week), cyclophosphamide monotherapy (q7d), or the combination, at the indicated dosages. Mean tumor volume ± SEM is plotted for n=12 per group.

Figure 30

Figure 30A

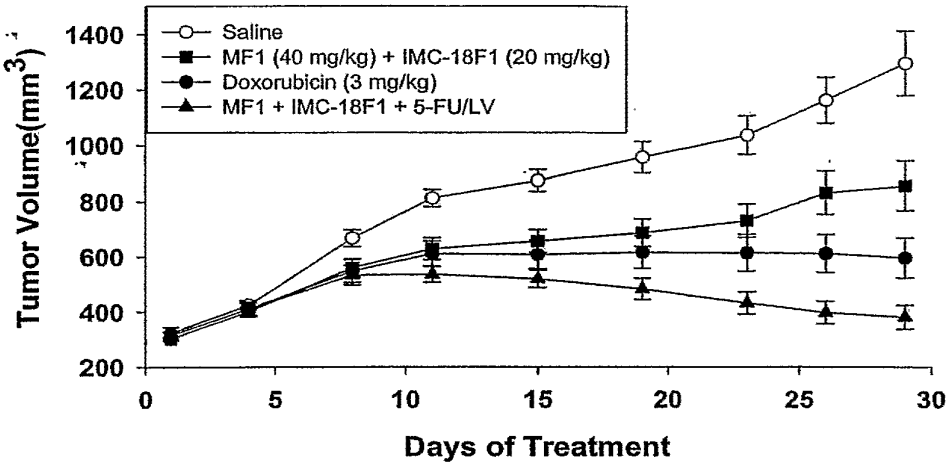


Figure 30B

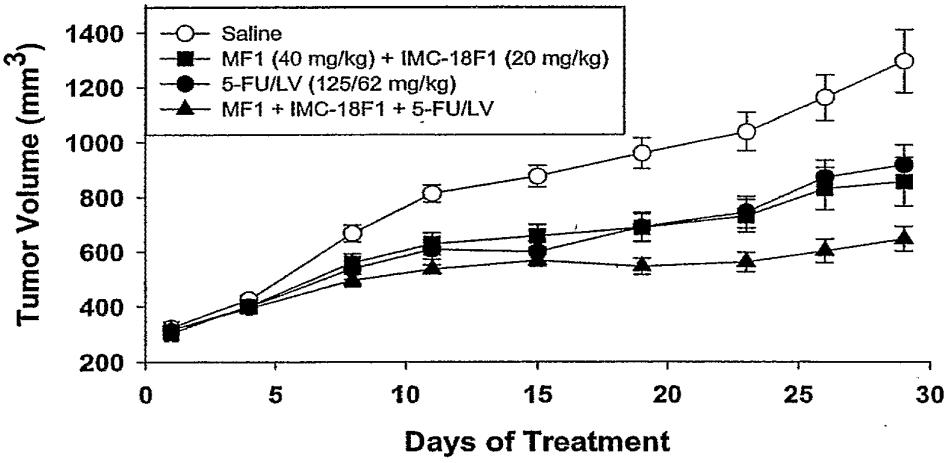


Figure 30A and **B**: Effect of 5-FU/LV or Doxorubicin Treatment in Combination with Anti-mouse and human VEGFR-1 Antibodies on the Growth of MDA-MB-231 Xenografts. Mice with MDA-MB-231 xenografts were treated with 18F1 + MF1 alone (3x/week), 5-FU/LV monotherapy (q7d) (A), doxorubicin (2x/week) (B) or a combination of antibody plus chemotherapy, at the indicated dosages. Mean tumor volume ± SEM is plotted for n=12 per group.

Figure 31

Figure 31A

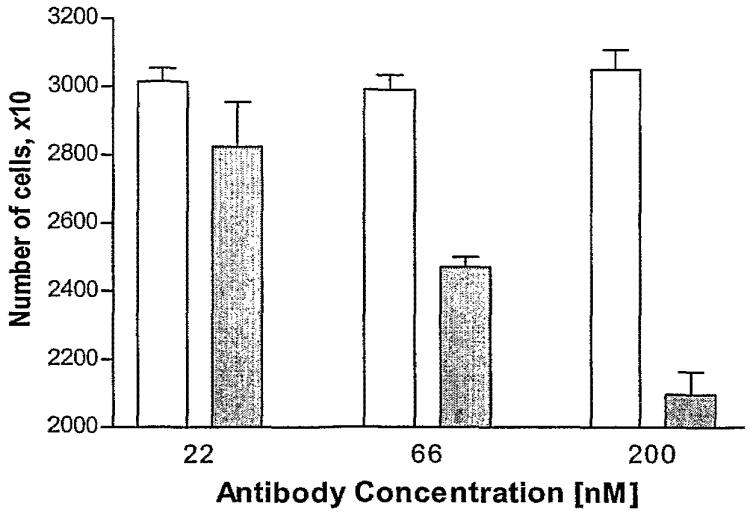


Figure 31B

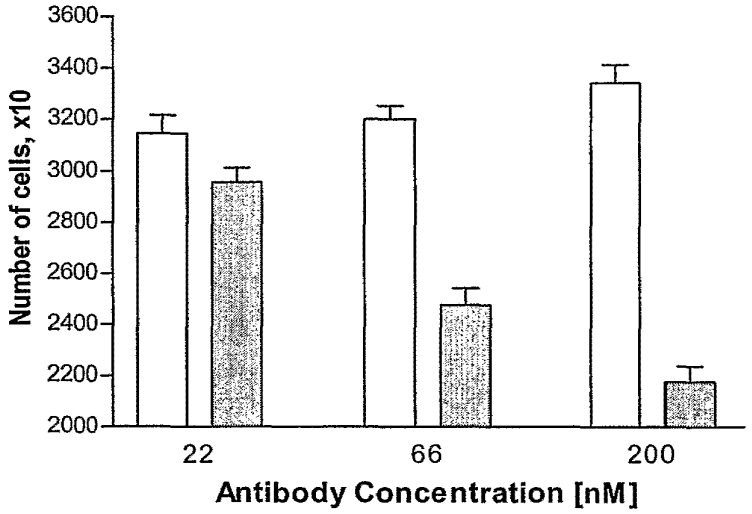


Figure 31A and **B:** Inhibition of Tumor Cell Proliferation. 2 x 10^4 DU4475 cells were serum starved overnight following treatment with desferrioxamine, then incubated with various amount of 18F1 (shadowed bar) or isotype control IgG (opened bar) in the presence of VEGF-A (A) or PlGF (B). After 2 days incubation, total cell number was determined using a Coulter cell counter. The results are shown as mean value with standard error.

Figure 32

Figure 32A

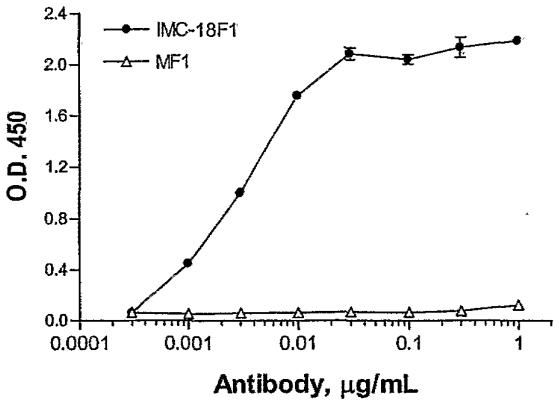


Figure 32B

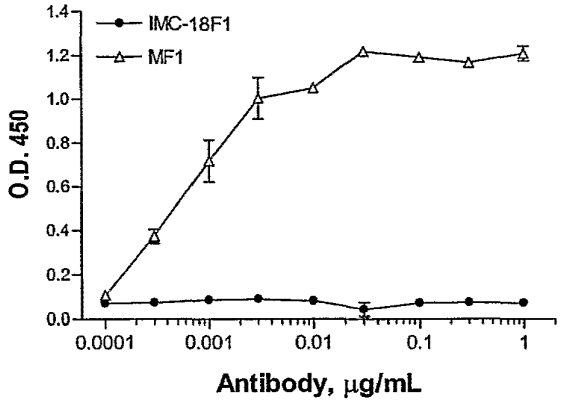


Figure 32C

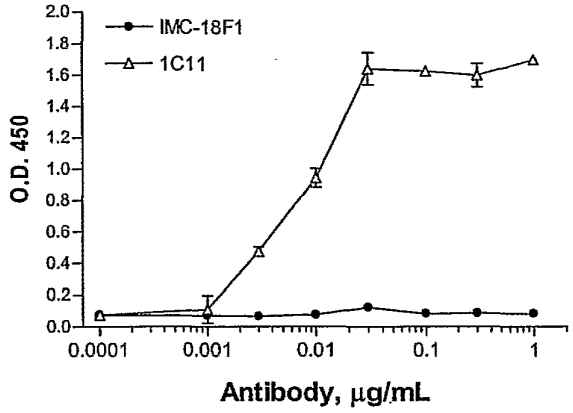


Figure 32A, B, and **C:** Specificity of 18F1 and Anti-mouse VEGFR-1 Antibody MF1. 18F1 has binding activity with immobilized recombinant human VEGFR-1 (A) but not with mouse VEGFR-1 (B) and human VEGFR-2 (C) as compared to positive control MF1 or VEGFR-2 specific antibody 1C11. Anti-mouse VEGFR-1 antibody MF1 did not bind to immobilized recombinant human VEGFR-1(A). 18F1 was used as a positive control in solid phase binding assay.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO: 13/149468
FILED: May 31, 2011
FOR: DOSAGE REGIMEN OF AN S1P RECEPTOR AGONIST

Art Unit: 1629
Examiner:

MS: Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Sir:

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO/SB/08A/B.

Copies of the references are enclosed herewith.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO/SB/08A/B form(s).

Please charge Deposit Account No. 19-0134 in the name of Novartis in the amount of $180 for payment of the fee pursuant to 37 CFR §1.17(p) for the submission of an Information Disclosure Statement under 37 CFR §1.97(c). The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Account No. 19-0134 in the name of Novartis.

Respectfully submitted,

Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

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One Health Plaza, Bldg. 101
East Hanover, NJ 07936
+1 862 7783785

Date: 7/19/12

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Hiestand, Peter C. et al.
APPLICATION NO: 13/149468
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Respectfully submitted,

Karen DeBenedictis
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Date: 7/19/12

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	1629
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
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Application Number:	13149468
Filing Date:	31-May-2011
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Filer:	Karen DeBenedictis/Denise Cooper
Attorney Docket Number:	PAT050279-US-CNT

Filed as Large Entity

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Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
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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:	12546461
Application Number:	13149468
International Application Number:	
Confirmation Number:	1536
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Customer Number:	1095
Filer:	Karen DeBenedictis/Denise Cooper
Filer Authorized By:	Karen DeBenedictis
Attorney Docket Number:	PAT050279-US-CNT
Receipt Date:	16-APR-2012
Filing Date:	31-MAY-2011
Time Stamp:	12:17:36
Application Type:	Utility under 35 USC 111(a)

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Payment was successfully received in RAM	$180
RAM confirmation Number	10438
Deposit Account	190134
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Warnings:					
Information:					
2		50279-IDS.pdf	110493 dcf72d3d17fa0091a95bf23fbedea6f0f0465958	yes	2

	Multipart Description/PDF files in .zip description		
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	Transmittal Letter	1	1
	Information Disclosure Statement (IDS) Form (SB08)	2	2

Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30482 2e9d06a9df16f834b2bdaf514249e5fd6299bbbf	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	181956		

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

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

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

With regard to other embodiments of the claimed invention, a medication for slowing progression of a demyelinating disease and for inhibiting or treating Primary-progressive multiple sclerosis, comprising an immunomodulator is known from the prior art (see RU 2199339 C2 27.02.2003) (D3).

Bibliographic data: RU2199339 (C2) — 2003-02-27

★ In my patents list ↗ Register → Report data error 🖶 Print

METHOD FOR TREATING MULTIPLE SCLEROSIS

Page bookmark	RU2199339 (C2) - METHOD FOR TREATING MULTIPLE SCLEROSIS
Inventor(s):	BARBAS I M; TOTOLJAN N A; SKOROMETS A A; SMIRNOV M N; ZHIVOTOVSKAJA M L; JAKOVLEVA V S ±
Applicant(s):	TEKH; OOO BIO ±
Classification:	- **international:** *A61K38/20*; (IPC1-7): A61K38/20
	- **European:**
Application number:	RU20010104929 20010223
Priority number(s):	RU20010104929 20010223

Abstract of RU2199339 (C2)

Translate this text into i
German Translate this text

FIELD: medicine, neurology. SUBSTANCE: during the stage of exacerbation patients with multiple sclerosis should be intravenously injected by drops with human interleukin-2 (h-IL-2) preparation, for example, roncoleukin, at the dosage of not less than 1 mln IU once per 7d. The method enables to considerably shorten therapy terms in patients with multiple sclerosis at the background of pronounced immune deficiency, decrease the number of relapses and prolong labor capacity period in patients with multiple sclerosis. EFFECT: higher efficiency of therapy. 1 cl, 2 ex, 2 tbl

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO: 13/149468
FILED: May 31, 2011
FOR: DOSAGE REGIMEN OF AN S1P RECEPTOR AGONIST

Art Unit: 1629
Examiner:

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Sir:

This paper is being filed:

☒ supplemental to the Information Disclosure Statements filed May 31, 2011 and December 30, 2011.

Please charge Deposit Account No. 19-0134 in the name of Novartis in the amount of $180 for payment of the fee pursuant to 37 CFR §1.17(p) for the submission of an Information Disclosure Statement under 37 CFR §1.97(c). The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Account No. 19-0134 in the name of Novartis.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO/SB/08A/B.

☒ Copies of the references are enclosed herewith.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO/SB/08A/B form(s).

Respectfully submitted,

Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936
+1 862 7783785

Date: 4/11/12

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/149,468	05/31/2011	Peter C. Hiestand	PAT050279-US-CNT	1536

1095 7590 04/03/2012

NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

EXAMINER	
SPIVACK, PHYLLIS G	
ART UNIT	**PAPER NUMBER**
1629	

MAIL DATE	DELIVERY MODE
04/03/2012	PAPER

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.

Office Action Summary	Application No.	Applicant(s)	
	13/149,468	HIESTAND ET AL.	
	Examiner	Art Unit	
	PHYLLIS G. SPIVACK	1629	

-- 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* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, 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 _____.

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) *12-21* 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) *12-21* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

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

a)☐ All b)☐ Some * c)☐ None of:

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *5/31/11; 9/29/11*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Applicants' Preliminary Amendment file May 31, 2011 is acknowledged. Claims 1-11 are canceled. New claims 12-21 are presented and represent all of the claims under consideration. A new Abstract and updated priority information are noted.

Information Disclosure Statements filed May 31, 2011 and September 29, 2011 are further acknowledged and have been reviewed. The reference to Dr. Maschkowskij cited on the Information Disclosure Statement filed September 29, 2011 is not present in the instant file or in the parent application.

The abstract of the disclosure is objected to because the subject matter under consideration is limited to administration of the compound 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-propane-1,3-diol, or a pharmaceutically acceptable salt thereof. The subject matter under consideration excludes prevention of neo-angiogenesis associated with a demyelinating disease. The terms depicted in formula X, i.e., Z, R_{1x}, R_{2Z} and R_{3Z} are not defined. Correction is required. See MPEP § 608.01(b).

Claims 18 and 19 are objected to under 37 CFR 1.75 as being substantial duplicates. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

Applicants are advised that should claim 18 be found allowable, claim 19 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof.

The disclosure is objected to for the following informality:

On page 12 of the specification, line 22, 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-propane-1,3-diol is misspelled.

Appropriate correction is required.

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

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims12-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Virley, D.J., Journal of the American Society for Experimental NeuroTherapeutics, in view of LaMontagne. et al., Cancer Research, and further in view of Kovarik et al., WO 06/058316.

Virley teaches the administration of 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-propane-1,3-diol, also known as FTY720, which is a sphingosine-1-phosphate receptor modulator, for the treatment of multiple sclerosis (MS). Virley distinguishes between the categories of relapsing-remitting MS and primary progressive MS. See the introduction on page 638, as well as the discussion of experimental models for MS on

page 640. In order to provide predictive indices for clinical application, experimental autoimmune encephalomyelitis (EAE) models are discussed.

Due to the majority of MS patients presenting relapsing-remitting symptoms before progressing onto a chronic phase, a number of animal models of EAE have been designed to simulate the more dynamic clinical and pathological features of relapsing-remitting MS. One such model, using the Biozzi AB/H mouse, involves the inoculation of homologous spinal cord homogenate (or more specifically MOG peptide) in adjuvant without the additional use of *Bordetella pertussis* toxin, and reproducibly induces a chronic relapsing-remitting demyelinating disease. The dynamic chronicity of symptoms is expressed as an acute induction of disease (loss of tail tone and hindlimb paralysis), followed by reduced severity (remission) and then a relapse disease episode. The development of clinical signs in this model are preceded by a loss in weight, whereas remission periods are associated with an increase in body weight, implicating changes in weight as surrogate markers of disease status. Reductions in the degree of inflammation and evidence for remyelination are thought to reflect the remission period in this EAE model, whereas relapses are thought to be indicative of an amplified inflammatory response, gliosis and demyelination within the CNS.

In particular, Virley teaches the oral administration of FTY720 in a Lewis rat EAE model demonstrates a dramatic reduction in clinical severity, mortality and the infiltration of leukocytes into the CNS. Proinflammatory cytokines such as IL-2, IL-6, and IFN's were markedly suppressed in the CNS following prophylactic FTY720 treatment relative to saline treatment. The mechanistic effect of FTY720 on reducing lymphocyte trafficking and CNS inflammation within EAE, was further studied with contrast-enhanced MRI, using superparamagnetic iron-oxide nanoparticles to track macrophage infiltration. The oral administration of FTY720 was shown to significantly reduce the magnitude and extent of cellular infiltration into the CNS of EAE-sensitized rats. These effects with FTY720 treatment corresponded to reductions in lesion burden and blood brain barrier disruption assessed by MRI signatures and a marked reduction in neurological disability during the acute and relapsing phase of the model. Therapeutic administration of FTY720 at the point of relapse also significantly suppressed further progression of clinical signs, providing compelling evidence for this

agent as a potential therapeutic agent for MS. Data from a phase II clinical trial with FTY720, confirmed a relapse reduction rate of more than 50% in 281 relapsing-remitting MS patients for 6 months of treatment, relative to placebo. Inflammatory disease activity, as visualized by gadolinium-enhanced T1-weighted MR imaging was shown to be dramatically reduced by up to 80%, after 6 months of oral, once a day, treatment. Progressive disease activity in the form of new T2 MRI lesions was also demonstrated to be reduced by more than 60% after FTY720 treatment. The onset of effect was demonstrated as early as 2 months following treatment, and MS patients showed no significant adverse events over the 6-month trial duration relative to placebo. See page 644.

Virley fails to teach the required dosage of 0.5 mg, as well as methods of inhibiting or treating a neo-angiogenesis association with MS.

However, LaMontagne teaches FTY720 to be an anti-angiogenic agent. The compound becomes phosphorylated *in vivo* and interacts with spingosine-1-phosphate (S1P) receptors. The effect is on vascular permeability, an important aspect of angiogenesis. See the Abstract.

Kovarik teaches dosage regimens involving S1P receptor agonists, of which FTY720 is clearly encompassed. Maintenance dosages are disclosed on page 15, line 9, as required by all of the present claims. Specifically, on line 16, page 17, a daily dose of 0.5 mg is taught for the treatment of autoimmune diseases, of which MS is recited as an example, on lines 7-8 on page 14.

Therefore, in view of the teachings of Virley, LaMontagne and Kovarik, one skilled in the neurology art would have been motivated to administer FTY720 with a reasonable expectation of success in inhibiting neo-angiogenesis associated multiple sclerosis, in alleviating relapses in MS and slowing the progression of MS. Such would have been obvious because FTY720 is a known anti-angiogenic agent that has been taught for use in the treatment of autoimmune diseases such as multiple sclerosis, at a dosage of 0.5 mg. According to Virley, FTY720 significantly reduced - by more than 60% - progressive disease activity after treatment. FTY720 administration resulted in a relapse reduction rate of more than 50% in 281 relapsing-remitting MS patients.

No claim is allowed.

Foster et al., US 2006/0046979, is cited to show further the state of the art.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Phyllis G. Spivack whose telephone number is 571-272-0585. The Examiner can normally be reached from 10:30 to 7 PM.

If attempts to reach the Examiner by telephone are unsuccessful after one business day, the Examiner's supervisor, Jeff Lundgren, can be reached 571-272-5541. 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).

March 24, 2012

/Phyllis G. Spivack/
Primary Examiner, Art Unit 1629

SEARCH HISTORY

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     FILE 'HCAPLUS' ENTERED AT 16:30:00 ON 21 MAR 2012
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L1           71 SEA ABB=ON  ("HIESTAND P C"/AU OR "HIESTAND PETER"/AU OR
                "HIESTAND PETER C"/AU)
                E SCHNELL CHRISTIAN/AU
L2           50 SEA ABB=ON  ("SCHNELL CHRISTIAN"/AU OR "SCHNELL
CHRISTIAN
                R"/AU OR "SCHNELL CHRISTIAN RENE"/AU)
L3            2 SEA ABB=ON  L1 AND L2
                SELECT RN L3 1-2

     FILE 'REGISTRY' ENTERED AT 16:30:28 ON 21 MAR 2012
L4            9 SEA ABB=ON  (162359-55-9/BI OR 507468-43-1/BI OR
800379-64-0/BI
                 OR 127464-60-2/BI OR 162359-56-0/BI OR 216974-75-3/BI
OR
                284461-73-0/BI OR 557795-19-4/BI OR 890128-81-1/BI)

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L5            2 SEA ABB=ON  L4 AND L3

     FILE 'REGISTRY' ENTERED AT 16:34:13 ON 21 MAR 2012
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L8          178 SEA SSS FUL L6
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L11           0 SEA ABB=ON  L10 AND C=33
L12           1 SEA ABB=ON  162359-55-9/RN
L13          38 SEA ABB=ON  L10 AND C=19
L14          35 SEA ABB=ON  L13 AND H=33
L15          20 SEA ABB=ON  L14 NOT D/ELS

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L17           1 SEA ABB=ON  L16 AND (NEO(W)ANGIOGENESIS OR
NEOANGIOGENESIS )
L18         258 SEA ABB=ON  L16 AND MULTIPLE SCLEROSIS
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L21           82 SEA ABB=ON  L19 AND S1P
L22          258 SEA ABB=ON  L19 OR L20 OR L21
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L24           68 SEA ABB=ON  L23 AND (?MULTIPLE?(W)?SCLEROSIS? OR
?ANGIOGENESIS?
                 )
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L28           68 SEA ABB=ON  L27 AND (?TREAT? OR ?THERAPY? OR ?INHIBIT?
OR
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L29           16 SEA ABB=ON  L28 AND ?RECEPT?(6A)?MODULAT?
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                 SAV L30 SPI468L30/A

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2012
L31           61 SEA ABB=ON  L26

     FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE' ENTERED AT 17:23:05 ON 21
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L32           55 DUP REMOV L26 L31 (17 DUPLICATES REMOVED)
L33            0 SEA ABB=ON  L32 AND (?DOSAGE? OR ?DOSE?)(5A)(0.5 OR
0.5(W) MG)



     FILE HOME

     FILE HCAPLUS

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PTO/SB/08a (07-09)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	2

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	Spivack
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
/P.S./		WO 2006/058316	06-01-2006			☐
/P.S./		WO 2004/113330	12-29-2004			☐
						☐
						☐
						☐
						☐

Examiner Signature	/Phyllis Spivack/	**Date Considered**	03/24/2012

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw a line through citation if not in conformance and not considered. Include copy of this form with the next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kind Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

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 2 hours 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, 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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Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT *(Use as many sheets as necessary)*				Application Number	
				Filing Date	
				First Named Inventor	Hiestand, Peter C. et al.
				Art unit	
				Examiner Name	
Sheet	2	of	2	Attorney Docket Number	PAT050279-US-CNT

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	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[2]
/P.S./		Brinkmann, Volker et al., "The Immune Modulator FTY720 Targets Sphingosine 1-Phosphate Receptors", The Journal of Biological Chemistry, Vol. 277, No. 24, Issue of June 14, pp. 21453-21457, (2002).	☐
/P.S./		Miller et al., Neurol, & Neurosci. Reports, (September, 2010), 1095), pp. 397-406	☐
/P.S./		Hla, T., FASEB Journal, (March 6, 2006), 20(4), part 1, A20.	☐
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Examiner Signature	/Phyllis Spivack/	Date Considered	03/24/2012

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CONFIRMATION NO. 1536

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
13/149,468	05/31/2011	514	1629	PAT050279-US-CNT

APPLICANTS

Peter C. Hiestand, Allschwil, SWITZERLAND;
Christian Schnell, Hesingue, FRANCE;

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

This application is a CON of 12/303,765 12/08/2008 ABN
which is a 371 of PCT/EP2007/005597 06/25/2007

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

UNITED KINGDOM 0612721.1 06/27/2006

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

06/15/2011

Foreign Priority claimed	☑ Yes ☐ No		STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	☑ Yes ☐ No	☐ Met after Allowance				
Verified and Acknowledged	/PHYLLIS G SPIVACK/ Examiner's Signature	Initials	SWITZERLAND	0	10	3

ADDRESS

NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080
UNITED STATES

TITLE

S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS

FILING FEE RECEIVED	FEES: Authority has been given in Paper	☐ All Fees
1480	No._____ to charge/credit DEPOSIT ACCOUNT	☐ 1.16 Fees (Filing)
	No._____ for following:	☐ 1.17 Fees (Processing Ext. of time)
		☐ 1.18 Fees (Issue)
		☐ Other _____
		☐ Credit

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	Spivack
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
/P.S./		US-2006/0046979	03-02-2006	Carolyn Ann Foster et al.	
		US-			
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
/P.S./		WO 2004/028521	04-08-2008	Novartis AG		☐
/P.S./		WO 2003/099192	12-04-2003	Novartis AG		☐
/P.S./		WO 2003/097028	11-27-203	Novartis AG		☐
/P.S./		WO 2004/050073	06-17-2004	Doosan Corporation		☐
/P.S./		WO 2005/123104				☐
						☐

Examiner Signature	/Phyllis Spivack/	Date Considered	03/24/2012

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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 2 hours 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, 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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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	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[2]
		~~Maochkowskij M.D. Pharmaceuticals, Moscow, Nowaja Wolna, 2001, in 2 volumes, vol. 1 p. 11~~	☐
/P.S./		LaMontagne K. „Antagonism of Sphingosine-1-Phosphate Receptors by FTY720 Inhibits Angiogenesis.." Cancer Research, Jan 2006, 66, 221-231. Found on:	☐
/P.S./		Hla. T. „Physiological and pathological actions of sphingosine 1-phosphate" Seminars in Cell & Developmental Biology, Oct 2004, 15(5), 513-520.	☐
/P.S./		Kappos L et al. „FTY720 in relapsing MS...." 23.06.2005 online (found 02.06.2011) URL:http://www.ms-in-europe.com/printversion/index.php?anr=105&cnr=4/>	☐
/P.S./		Ho J.W et al. „ Effects of a novel immunomodulating agent.." Molecular cancer theraputics, 2005 Set, 4(9), 1430-1438.Found:	☐
/P.S./		Virely D.J. "Developing therapeutics for the treatment of multiple sclerosis." Journal of American Society for Experimental Neuro Therapeutics. Oct 2005, 2, 638-649. Found on: http://pubget.com/paper/16489371	☐
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Examiner Signature	/Phyllis Spivack/	Date Considered	03/24/2012

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Search Notes	Application/Control No.	Applicant(s)/Patent under Reexamination	
	13/149,468	HIESTAND ET AL.	
	Examiner	Art Unit	
	PHYLLIS G. SPIVACK	1629	

SEARCHED			
Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
STN & inventors search	3/21/12	PS
PALM inventors search	3/24/12	PS

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/149,468	05/31/2011	Peter C. Hiestand	PAT050279-US-CNT

CONFIRMATION NO. 1536

1095
NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

PUBLICATION NOTICE

Title:S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS

Publication No.US-2011-0237682-A1
Publication Date:09/29/2011

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.

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Approved for use through 07/31/2012. OMB 0651-0031
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Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-2006/0046979	03-02-2006	Carolyn Ann Foster et al.	
		US-			
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		WO 2003/099192	12-04-2003	Novartis AG		☐
		WO 2003/097028	11-27-203	Novartis AG		☐
		WO 2004/050073	06-17-2004	Doosan Corporation		☐
		WO 2005/123104				☐
						☐

Examiner Signature		**Date Considered**	

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PTO/SB/08b (07-09)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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		Maschkowskij M.D. Pharmaceuticals, Moscow, Nowaja Wolna, 2001, in 2 volumes, vol.1 p.11	☐
		LaMontagne K. „Antagonism of Sphingosine-1-Phosphate Receptors by FTY720 Inhibits Angiogenesis.." Cancer Research, Jan 2006, 66, 221-231. Found on:	☐
		Hla. T. „Physiological and pathological actions of sphingosine 1-phosphate" Seminars in Cell & Developmental Biology, Oct 2004, 15(5), 513-520.	☐
		Kappos L et al. „FTY720 in relapsing MS...." 23.06.2005 online (found 02.06.2011) URL:http://www.ms-in-europe.com/printversion/index.php?anr=105&cnr=4/>	☐
		Ho J.W et al. „ Effects of a novel immunomodulating agent.." Molecular cancer theraputics, 2005 Set, 4(9), 1430-1438.Found:	☐
		Virely D.J. "Developing therapeutics for the treatment of multiple sclerosis." Journal of American Society for Experimental Neuro Therapeutics. Oct 2005, 2, 638-649. Found on: http://pubget.com/paper/16489371	☐
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If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

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

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0212210.9	27 May 2002 (27.05.2002)	GB
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(71) Applicant *(for all designated States except AT, US)*: **NOVARTIS AG** [CH/CH]; Lichtstrasse 35, CH-4056 Basel (CH).

(71) Applicant *(for AT only)*: **NOVARTIS Pharma GmbH** [AT/AT]; Brunner Strasse 59, 1230 Vienna (AT).

(71) Applicant *(for all designated States except US)*: **IRM LLC** [—/—]; P.O. Box HM 2899, Hamilton, HM LX (BM).

(72) Inventors; and

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(74) Agent: **GROS, Florent**; Novartis AG, Corporate Intellectual Property, CH-4002 Basel (CH).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LT, LU, LV, MA, MD, MK, MN, MX, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SE, SG, SK, TJ, TM, TN, TR, TT, UA, US, UZ, VC, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR).

Published:
— *without international search report and to be republished upon receipt of that report*

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

(54) Title: BIS-AROMATIC ALKANOLS

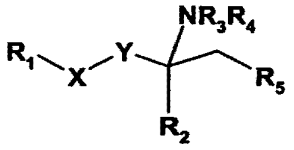
(57) Abstract: The present invention relates to biphenylyl derivatives, processes for their production, their uses and pharmaceutical compositions containing them.

WO 03/099192 A2

Bis-aromatic Alkanols

The present invention relates to biphenylyl derivatives, processes for their production, their uses and pharmaceutical compositions containing them.

More particularly the present invention provides a compound of formula I



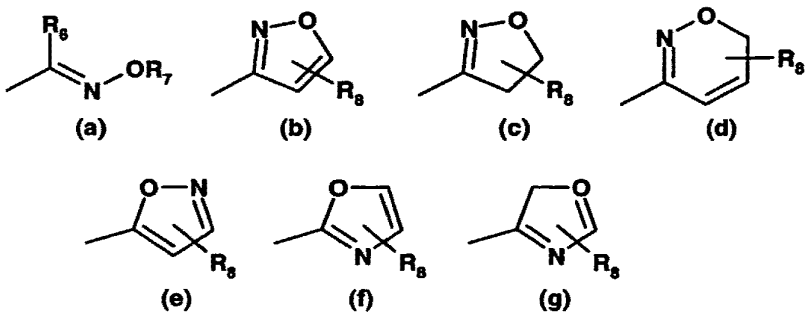
I

wherein

Y is $-CH_2CH_2-$, $-CH_2CH(OH)-$, $-CH(OH)CH_2-$, $-C(O)CH_2-$, $-CH_2C(O)-$, $-CH=CH-$; or 1,2-cyclopropylene;

X is arylene or C_{5-6}heteroarylene optionally substituted by one to three substituents selected from halogen, nitro, C_{1-10}alkyl and halogen-substituted C_{1-6}alkyl;

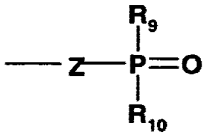
R_1 is aryl, aryl-C_{2-4}alkenyl, heteroaryl, or heteroaryl-C_{2-4}alkenyl each being substituted by (i) one to three substituents selected from hydrogen, halogen, amino, phenyl, heteroaryl, heteroaryl-C_{1-4}alkyl, C_{1-10}alkyl, cycloalkyl-C_{1-4}alkyl, cycloalkyl-C_{1-4}alkoxy, C_{1-10}alkoxy, C_{2-10}alkenyl, C_{2-10}alkynyl, C_{1-10}alkylthio, C_{1-10}alkylsulfonyl, C_{1-10}alkyl-sulfinyl, C_{1-4}alkyl-$S(O)_2NH$-, phenylC_{1-6}alkyl, or phenylC_{1-6}alkoxy, in each of which any aliphatic part of the group may be straight or branched chain and optionally substituted by up to three substituents selected from halogen, amino, hydroxy, cyano, or cycloalkyl groups and optionally interrupted by a double or triple bond or one or more C(O), NR_{12}, S, S(O), $S(O)_2$ or O groups, wherein R_{12} is hydrogen or C_{1-6}alkyl; and any aromatic group may be optionally substituted by one to three substituents selected from halogen, cyano, amino, C_{1-4}alkyl halogen-substituted-C_{1-4}alkyl and C_{1-8}alkoxy; and/or (ii) a group of formula (a), (b), (c), (d), (e), (f) or (g):



in which each of

R_6, R_7 and R_8 independently, is hydrogen; phenyl, C_{1-10}alkyl, cycloalkyl, heteroaryl, heteroaryl-C_{1-4}alkyl, C_{1-10}alkoxy, C_{2-10}alkenyl, C_{2-10}alkynyl, C_{1-10}alkylthio, C_{1-10}alkylsulfonyl, C_{1-10}alkylsulfinyl, phenylC_{1-8}alkyl, or phenylC_{1-6}alkoxy, in each of which any aliphatic part of the group may be straight chain or branched and may be optionally substituted by up to three halogen, hydroxy, cycloalkyl, or C_{1-4}alkoxy groups and optionally interrupted by a double or triple bond or one or more C(O), NR_{12}, S, S(O), $S(O)_2$ or O groups, and any aromatic group may be optionally substituted by one to three substituents selected from halogen, CF_3, C_{1-8}alkyl and C_{1-8}alkoxy;

R_2 is hydrogen; halogen; C_{1-4}alkyl optionally substituted with one or more halogens; C_{2-6} alkenyl; C_{2-6}alkynyl; cycloalkyl optionally substituted by halogen; aryl optionally substituted with hydroxy; or C_{1-4}alkyl optionally substituted on the terminal C atom by OH or a residue of formula (h):



(h)

in which Z is a direct bond, O, S, $(CH_2)_{1-2}$, CF_2, or NR_{11} where R_{11} is H, (C_{1-4})alkyl or halogen-substituted (C_{1-4})alkyl; and each of R_9 and R_{10}, independently, is H, OH, (C_{1-4})alkyl optionally substituted by one to three halogens, or (C_{1-4})alkoxy optionally substituted by halogen; with the proviso that R_9 and R_{10} are not both hydrogen;

each of R_3 and R_4, independently, is H or C_{1-4}alkyl optionally substituted by halogen or acyl; and R_5 is H, –OH, -Oacyl, –NHacyl, or a residue of formula (h) as defined above;

provided that at least either R_2 comprises a terminal OH or a residue of formula (h) or R_5 is OH or a residue of formula (h),

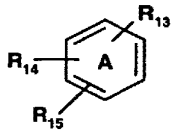
or a salt thereof.

Alkyl as a group and as a structural element of other groups, e.g. halogen-substituted-alkyl, alkoxy, acyl, alkylthio, alkylsulfonyl and alkylsulfinyl, may be straight or branched chain, e.g. methyl, ethyl, propyl, iso-propyl or butyl. Alkenyl as a group and as a structural element of other groups contains one or more carbon-carbon double bonds and may be e.g. vinyl. Any double bonds may be in the cis- or trans- configuration. Alkynyl as a group and as a structural element of other groups and compounds contains at least one carbon – carbon triple bond and may also contain one or more C=C double bonds, and may be e.g. propyn-2-yl. Alkyl, alkenyl, alkynyl or cycloalkyl substituted by halogen, e.g. as R_2, may be alkyl, alkenyl, alkynyl or cycloalkyl wherein one or more H are replaced by halogen, e.g. Cl or F, e.g. $CHCl\text{-}CH_3$ or CF_3; halogen-substituted alkyl, alkenyl, alkynyl or cycloalkyl may be partially halogenated or perhalogenated, whereby in the case of multiple halogenation, the halogen substituents may be identical or different.

Any cycloalkyl group, alone or as a structural element of other groups may contain from 3 to 8 carbon atoms, e.g. from 3 to 7 carbon atoms, preferably from 3 to 6 carbon atoms.

Acyl may be a residue R-CO wherein R is C_{1-6}alkyl, C_{3-6}cycloalkyl, phenyl or phenylC_{1-4}alkyl. Halogen may be F, Cl or Br, preferably F or Cl.

Aryl means a monocyclic or fused bicyclic aromatic ring assembly, e.g. containing six to ten ring carbon atoms. For example aryl may be naphthyl, phenyl, or phenyl optionally substituted, preferably a residue of formula (k):



(k)

wherein each of R_{13}, R_{14} and R_{15}, independently, is H; halogen; C_{1-8}alkyl optionally substituted by one or more halogen, hydroxy, or C_{1-4}alkoxy or optionally interrupted by one oxy or by one or more oxygen atoms; C_{1-8}alkoxy; C_{2-8}alkenyl; C_{2-8}alkynyl; C_{1-8}alkylthio; C_{1-8}alkylsulfonyl; C_{1-8}alkylsulfinyl; phenylC_{1-6}alkyl; phenylC_{1-6}alkoxy; phenyl optionally substituted by halogen, CF_3, C_{1-4}alkyl and/or C_{1-4}alkoxy. When ring A is monosubstituted, the substitutent is preferably in the *para* position.

Arylene means a divalent radical derived from an aryl group. For example arylene as used in this application may be phenylene or naphthylene, preferably phenylene, more preferably 1,4-phenylene.

Aryl-C_{2-4}alkenyl may be e.g. styryl.

Heteroaryl means aryl, as defined in this application, optionally substituted, provided that one or more of the ring carbon atoms indicated are replaced by a heteroatom, e.g. 1 to 3 heteroatoms, selected from N, O or S, and, e.g. each ring is comprised of 5 to 9 ring atoms. Examples include thienyl, pyridinyl, isoxazolyl, benzoxazolyl, benzo[1,3]dioxolyl, furyl, pyrrolyl, benzothienyl, benzofuryl, indolyl or benzoxadiazolyl, preferably thienyl or pyridinyl. Suitable substitutents are e.g. methyl, halogen or formyl. When substituted, it is preferably monosubstituted. Heteroarylene means heteroaryl, as defined in this application, provided that the ring assembly comprises a divalent radical.

Compounds of the present invention are often active with free hydroxy and free amine groups. Forms of the compound that have the hydroxy or amine group present in a protected form often function as prodrugs. Prodrugs are compounds that are converted into an active drug form after administration, through one or more chemical or biochemical transformations. Forms of the compounds of the present invention that are readily converted into the claimed compound under physiological conditions are prodrugs of the claimed compounds and are within the scope of the present invention. Examples of prodrugs include forms where a hydroxy group is acylated to form a relatively labile ester such as an acetate ester, and forms where an amine group is acylated with the carboxylate group of glycine or an L-amino acid such as serine, forming an amide bond that is particularly susceptible to hydrolysis by common metabolic enzymes. Some molecules of the present invention may themselves be prodrugs, such as those comprising a phosphate residue of formula (h) which may be enzymatically dephosphorylated to a hydroxy group. Alternatively, a compound of the invention wherein R_2 and/or R_5 comprises a free hydroxy group may be enzymatically phosphorylated to a compound comprising a phosphate residue of formula (h). The present invention also includes both the enzymatically phosphorylated or dephosphorylated compounds of formula I, optionally in equilibrium.

Compounds of formula I may exist in free form or in salt form, e.g. addition salts with e.g. inorganic acids, such as hydrochloride, hydrobromide or sulfate, salts with organic acids, such as acetate, fumarate, maleate, benzoate, citrate, malate, methanesulfonate or benzenesulfonate salts; when group (h) is present and R_9 or R_{10} is –OH, group (h) may also be present in salt form, e.g. an ammonium salt or salts with metals such as sodium, potassium, calcium, zinc or magnesium, or a mixture thereof. Compounds of formula I and their salts in hydrate or solvate form are also part of the invention.

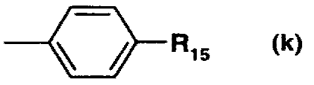
When the compounds of formula I have asymmetric centers in the molecule, various optical isomers are obtained. The present invention also encompasses enantiomers, racemates, diastereoisomers and mixtures thereof. For example the central carbon atom bearing R_2, CH_2-R_5 and NR_3R_4 may have the R or S configuration. Compounds having the R configuration at this central carbon atom are preferred. Moreover, when the compounds of formula I include geometric isomers, the present invention embraces cis-compounds, trans-compounds and mixtures thereof. Similar considerations apply in relation to starting materials exhibiting asymmetric carbon atoms or unsaturated bonds as mentioned above.

In the compounds of formula (I), the following significances are preferred individually or in any sub-combination:

1. Y is $-CH_2-CH_2-$ or $-CH(OH)-CH_2-$, preferably $-CH_2-CH_2-$;

2. X is 1,4-phenylene;

3. R_1 is mono- or di-substituted phenyl or thienyl, preferably para-monosubstituted phenyl, e.g. substituted by a group R_{15}, as defined below; e.g. R_1 is a group of formula (k)



(k)

where R_{15} is straight chain C_{5-8}alkyl; C_{2-8}alkenyl; or straight chain or branched C_{1-8}alkoxy optionally substituted by one C_{3-6}cycloalkyl or by a phenyl group optionally substituted by up to three halogens;

4. R_1 is mono- or di-substituted phenyl or thienyl, preferably para-monosubstituted phenyl, e.g. substituted by a group of formula (a), (b) or (c) as defined above;

5. R_1 is phenyl monosubstituted by a group of formula (a), preferably in the trans configuration;

6. In the group of formula (a), R_6 is C_{1-6}alkyl or C_{3-6}cycloalkyl, preferably straight chain C_{1-4}alkyl, cyclopropyl or cyclopropylmethyl;

7. In the group of formula (a), R_7 is H, C_{1-6}alkyl, C_{2-6}alkenyl, or C_{2-6}alkynyl, preferably straight chain C_{1-6}alkyl, vinyl, allyl or propyn-2-yl;

8. R_2 is C_{1-4}alkyl optionally substituted on the terminal C atom by OH or a residue of formula (h), preferably R_2 is methyl or hydroxymethyl, more preferably hydroxymethyl;

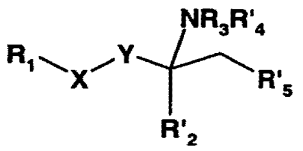
9. At least one of R_3 and R_4 is hydrogen, preferably both are hydrogen;

10. R_5 is hydrogen, –OH, –NHC(O)C_{1-4}alkyl or a residue of formula (h);

11. Each of R_9 and R_{10} is –OH;

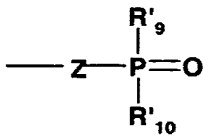
12. Z is O.

The present invention also includes a process for the preparation of a compound of formula I which process comprises removing the hydrolysable groups present in a compound of formula II



II

wherein X, Y, R_1 and R_3 are as defined above, R_4' is an amino protecting group, R_2' has one of the significances given for R_2 above except that the terminal OH when present in the OH-substituted C_{1-4}alkyl is in protected form or the residue of formula (h) is replaced by a residue of formula (h') and R_5' is R_5" in which R_5" is H, -OH in protected form or a residue of formula (h'), provided that at least one of R_2' and R_5' is OH in protected form or a residue of formula (h'), the residue of formula (h') being:



(h')

wherein Z is as described above, and each of R_9' and R_{10}' is a hydrolysable group and, where required, converting the compounds of formula I obtained in free form into the desired salt form, or vice versa.

The process may be carried out in accordance with methods known in the art. Hydrolysable groups may be hydroxy and amino protecting groups, e.g. when compounds of formula I are free of a residue of formula (h), and/or groups such as R'_9 and R'_{10}. Examples of protecting groups for hydroxy and amino groups are, e.g. as disclosed in "Protective Groups in Organic Synthesis" T.W. Greene, J. Wiley & Sons NY, 2nd ed., chapter 7, 1991, and references therein, e.g. benzyl, p-methoxybenzyl, methoxymethyl, tetrahydropyranyl, trialkylsilyl, acyl, *tert*-butoxy-carbonyl, benzyloxy-carbonyl, 9-fluorenylmethoxycarbonyl, trifluoroacetyl, trimethylsilyl-ethanesulfonyl and the like.

Preferably R_9' and R_{10}' are identical and have the significance of, e.g. phenoxy or benzoxy or form together a cyclic system such as in 1,5-dihydro-2,4,3-benzodioxaphosphepin.

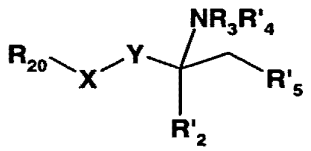
The removal of the hydroxy and amino protecting groups and/or of R'_4 or R'_5 groups in the compounds of formula II may conveniently be performed according to methods known in the art, e.g. by hydrolysis, e.g. in a basic medium, e.g. using a hydroxide such as barium hydroxide. It may also be performed by hydrogenolysis, e.g. in the presence of Pearlman's catalyst, e.g. as disclosed in J. Org. Chem., 1998, 63, 2375-2377. When the compounds of formula II are free of a residue of formula (h'), the removal of the hydroxy and amino protecting groups may also be performed in an acidic medium.

Compounds of formula II, used as starting materials, and salts thereof are also novel and form part of the invention.

The present invention also includes a process for the preparation of a compound of formula II which process comprises coupling a compound of formula III:

$$R_1—Q \qquad \text{III}$$

wherein R_1 is as defined above, Q is boron, silicon, magnesium, tin, lithium, copper or zinc, where each of these elements is bound to one or more suitable ligands, e.g. hydroxy, C_{1-8}alkoxy, C_{1-8}alkyl optionally substituted by a terminal carboxyl group, halogen or pseudohalogen, e.g. triflate (trifluoromethylsulfonate), mesylate, tosylate or cyanide; with a compound of formula IV:



IV

wherein X, Y, R'_2, R_3, R'_4 and R'_5 are as specified above, and R_{20} is halogen, preferably Cl, Br, I, triflate, tosylate or mesylate;

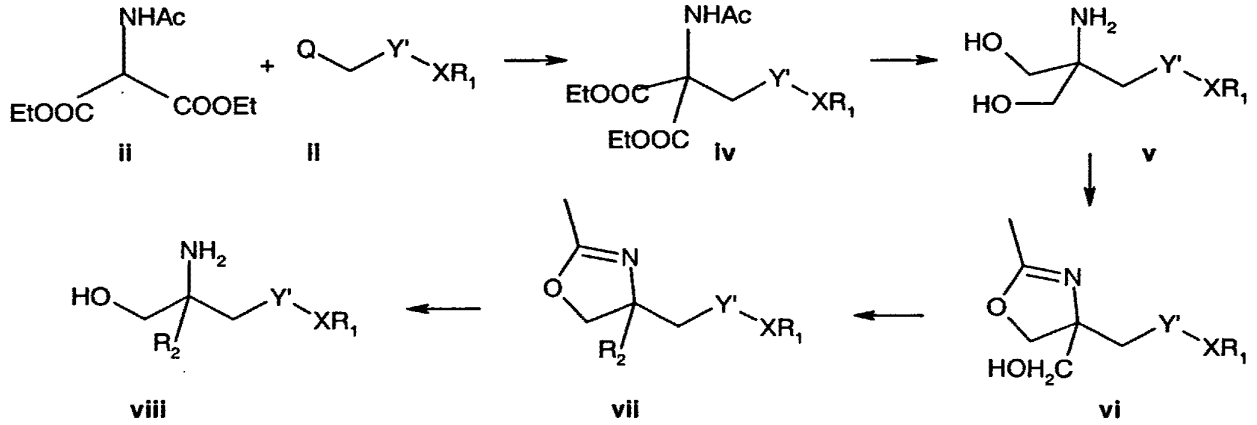
under the catalysis of a transition metal or salt thereof, e.g. palladium, rhodium or platinum, e.g. in the presence of a suitable ligand, e.g. a phosphine, carboxylate or heterocyclic carbene.

Compounds of formula II wherein R'_5 is a residue of formula (h') may also be prepared by reacting a compound of formula II wherein R'_5 is hydroxy in protected or unprotected form, with a corresponding phosphorylating agent, e.g. a phosphorochloridate, e.g. diphenylchlorophosphate or dibenzylchlorophosphate, cyanoethylphosphate, a phosphoramidate such as

N-phenyl phosphoramidate, 3-(diethylamino)-1,5-dihydro-2,4,3-benzodioxaphosphepin and the like.

Many compounds of the present invention, having general structure of Formula I, may be synthesized from protected aminomalonate esters such as ii (Scheme I). This compound may be readily alkylated by alkylating agents such as iii (wherein Y' is CH_2, CH(OH) or C=O) having leaving groups (-Q) such as bromide, iodide, or an alkyl or aryl sulfonate ester. These alkylating agents and methods for their preparation are generally well known in the art. The products of these alkylations are compounds such as iv, which may be reduced to produce compounds v of the present invention. This approach enables synthesis of compounds v, having various X-R_1 groups and linking groups between X and the aminopropanediol.

Scheme I.

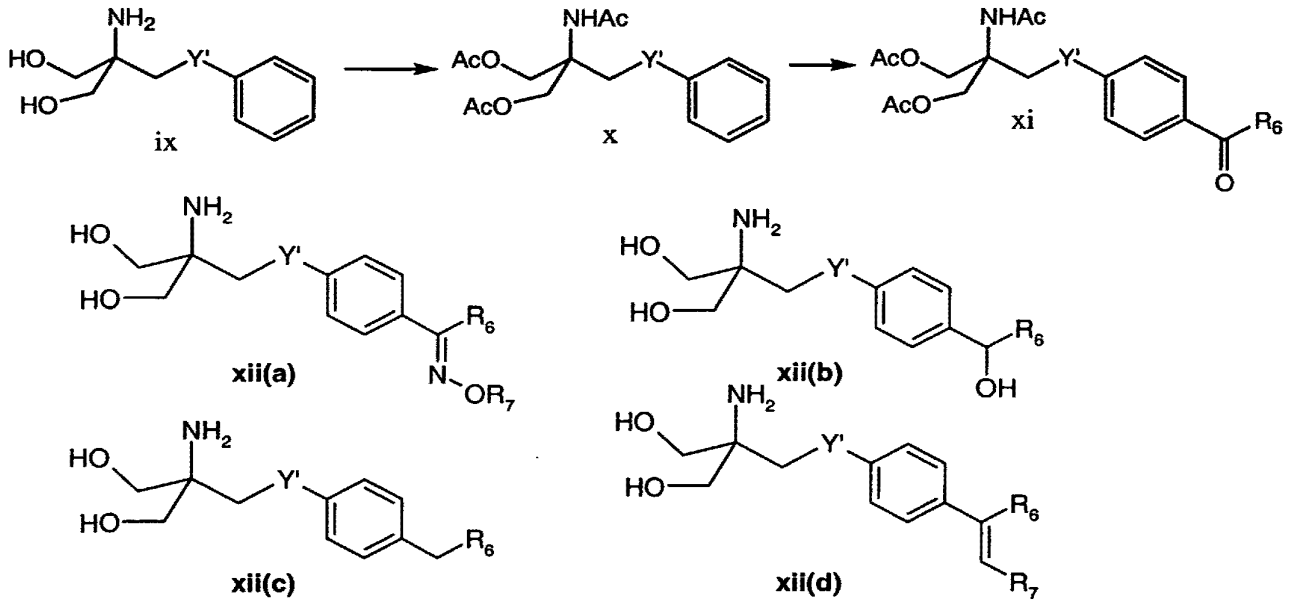


Compounds such as v may be used to prepare other compounds of the present invention, using well known protection strategies (Scheme I) to differentiate the two hydroxy groups. For example, v may be protected as an oxazoline (vi), leaving one hydroxy group free for further functionalization. Methods well known in the art (alkylation, acylation, oxidation, reduction, and combinations of these steps) may be used to convert the CH_2OH group of compound vi into various R_2 groups to provide other compounds that are within the scope of the present invention such as vii and viii.

Alternatively, a compound of formula ix may be protected by acylation, for example, allowing functionalization of the aryl group (Scheme II, wherein Y', R_6 and R_7 are as described above). When the aryl group is a phenyl, as shown for example with ix, it may be acylated to produce a compound x that may undergo Friedel-Crafts acylation under conventional conditions to produce a compound such as xi. This acylated compound may then be further transformed into compounds such as xii(a), xii(b), xii(c) and xii(d) by procedures well known

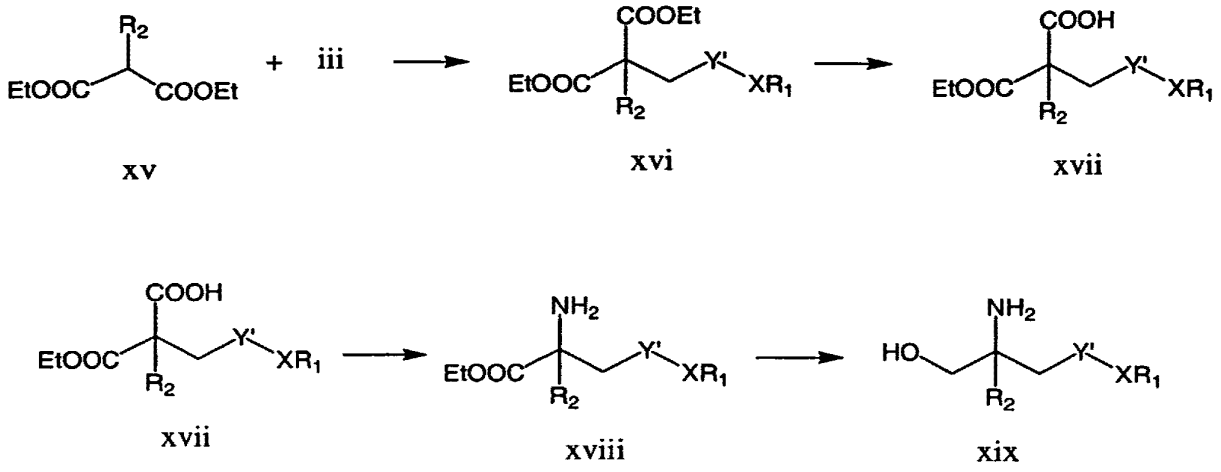
to those of skill in the art. For example, conversion to an oxime (xii(a)) is accomplished by treatment with an alkoxyamine as described below for Example 5. Reduction to the alcohol xii(b) may be accomplished with sodium borohydride, for example; further reduction to remove the hydroxy group (producing xii(c)) may be achieved with catalytic hydrogenation or with triethylsilane and trifluoroacetic acid. Olefination to form xii(d) may be achieved with Wittig or Horner-Emmons conditions, via Petersen olefination, or by a Grignard addition followed by elimination of the benzylic alcohol. Such transformations enable the incorporation of diverse substituents on the aryl groups of the compounds of the present invention.

Scheme II



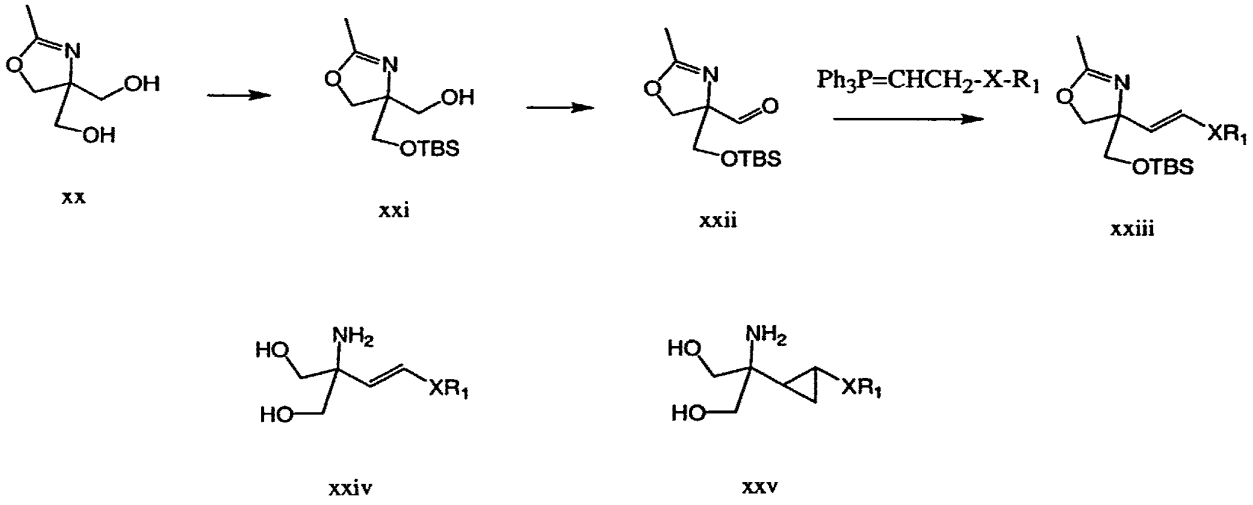
Another general method for preparing compounds where R_2 is other than CH_2OH or a residue of formula (h) (referred to herein as R''_2), begins with a malonate ester such as xv, which may be alkylated with an alkylating agent such as iii (Scheme III, wherein Y' is as described above). This provides intermediate xvi, which may be selectively hydrolyzed under conditions known in the art to give xvii. Compounds of this general structure may be converted to an amide or acyl azide, for example, which may be used to prepare compounds xix. Reduction of the ester group then provides compounds xix of the present invention. This enables access to compounds wherein R_2 is an aryl group such as e.g. 2-hydroxyphenyl.

Scheme III



Another versatile method for preparing compounds of the present invention utilizes xx, which is a known compound that may be selectively protected as xxi and oxidized to provide xxii (Scheme IV, wherein Y' is as described above). Aldehyde xxii may be used for a Wittig olefination reaction, e.g. to produce xxiv. This compound, after deprotection, may provide compounds xxv of the present invention. Alternatively, it may be used to synthesize other compounds such as e.g. compound xxvi, which is produced by cyclopropanation of the olefin of xxiv followed by deprotection.

Scheme IV

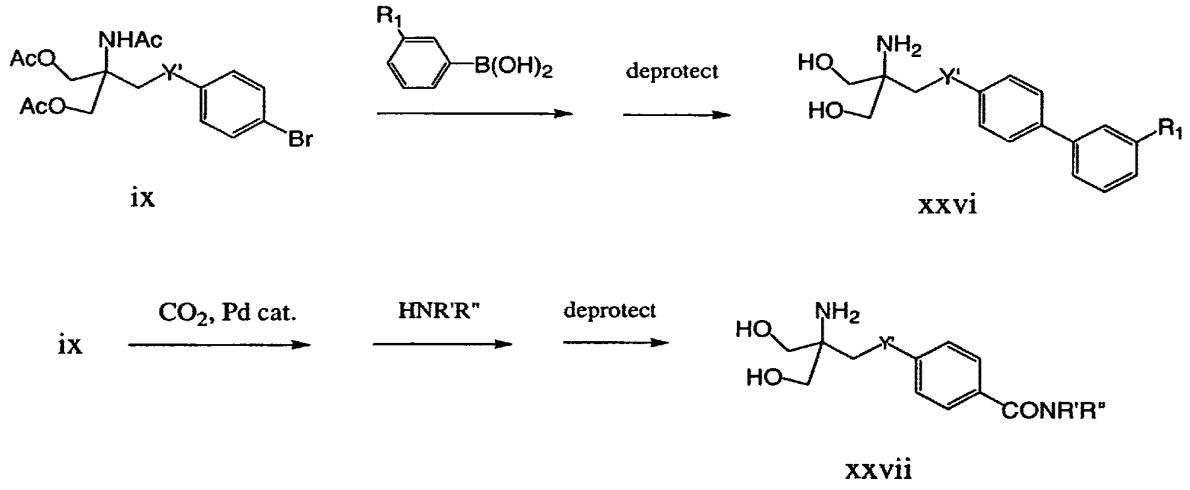


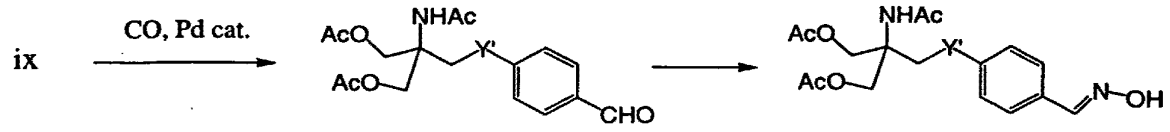
Certain compounds from the above schemes serve as versatile intermediates that allow further functionalization of the X group (Scheme V, wherein Y' is as described above). For

example, compounds such as iv, x, xvi, and xxiii have their hydroxy and amine groups protected; when X in such compounds is X' containing certain functional groups, they may be used to introduce new features on X. For example, if X' is a bromophenyl, bromopyridyl or similar group suitable for Suzuki reactions and similar palladium-catalyzed coupling reactions, X' may be arylated to provide biaryl compounds of the present invention, e.g. compounds of Formula I containing a biaryl group, like xxvi.

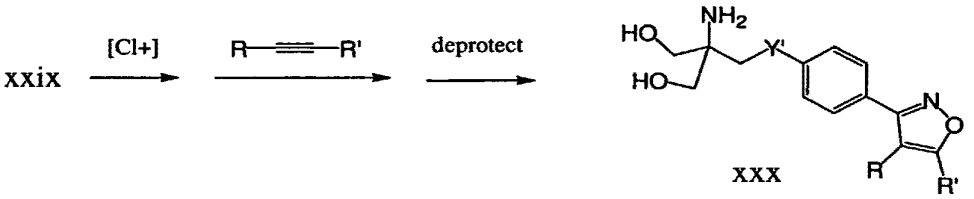
Alternatively, such bromophenyl and similar compounds may be carboxylated in the presence of a palladium catalyst and CO_2, and the carboxyl group may be used to introduce features such as an amide group. Furthermore, such bromophenyl and similar compounds may be carbonylated in the presence of palladium catalyst and CO, to introduce an aldehyde group. The aldehyde may then be used e.g. in Grignard or Wittig reactions to introduce new alkyl or aryl groups, or it may e.g. be converted into an oxime by reaction with hydroxylamine. Oximes such as xxix may be used to generate nitrile oxide intermediates by procedures well known in the art, and these readily undergo [3+2] cycloaddition reactions with olefins and acetylenes to produce isoxazolines and isoxazoles, respectively.

Scheme V





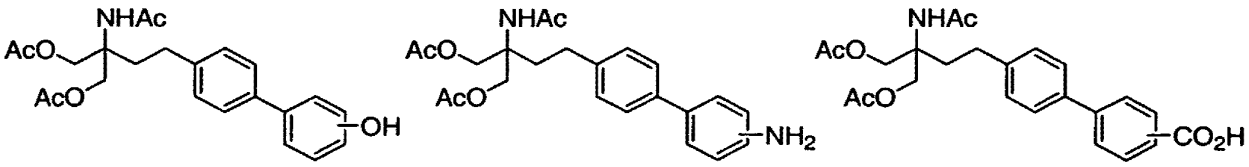
xxviii xxix



xxx

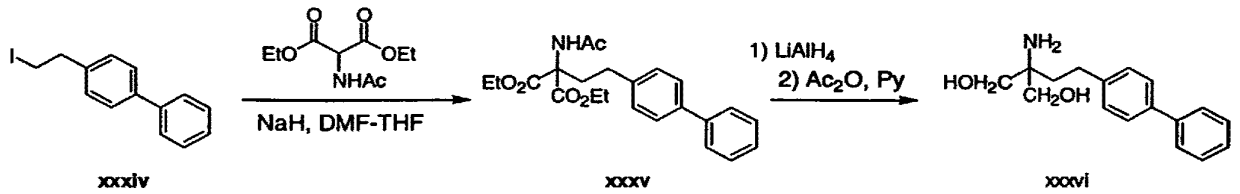
Furthermore, using protected intermediates such as xxiii above, an aryl ring X may be converted readily by methods known in the art to an arylboronic acid or an aryltrimethyltin species that may be used in Suzuki or Stille type coupling reactions to produce other biaryl compounds of the present invention.

Alternatively, a starting compound wherein X contains a nitro group as a substituent, that group may be reduced and alkylated, acylated or sulfonylated to produce other compounds of the present invention. A hydroxy group present in protected form may be deprotected and alkylated or otherwise modified, including being converted into a trifluoromethylsulfonate ("triflate") or similar functional group that is useful for palladium-catalyzed replacement reactions. Other substituents may likewise be incorporated on the aryl groups of intermediates such as those illustrated, as those of skill in the art will appreciate, and may also be transformed using well known methods into other groups to provide other compounds of the present invention. Examples of some very versatile intermediates of this type are shown below:



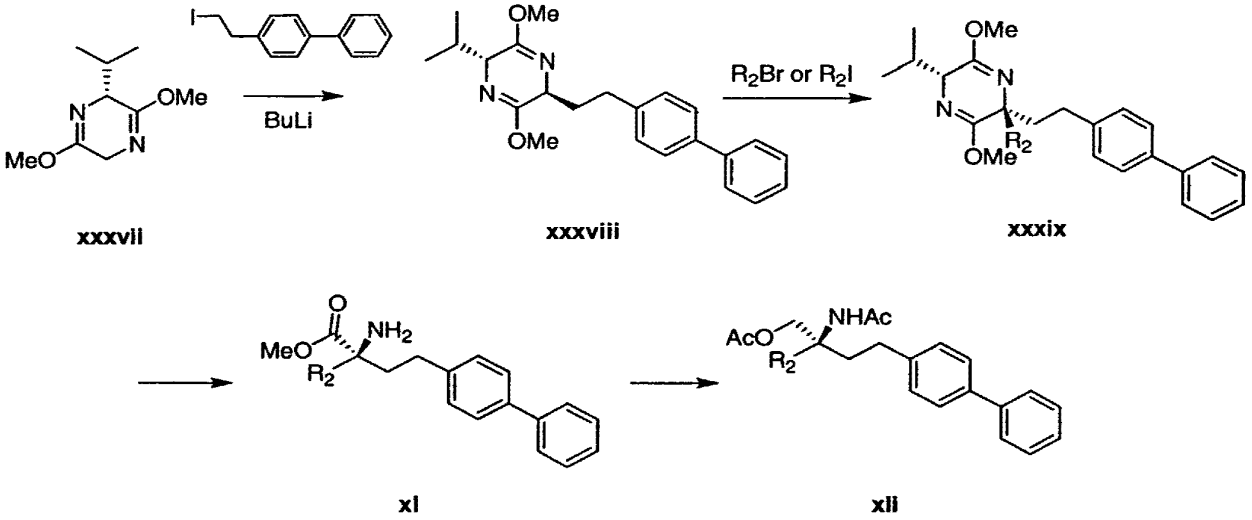
xxxi xxxii xxxiii

Certain key intermediates that are particularly useful for practicing the present invention are known in the art. For example, compound xxxvi, the preparation of which is described by Kiuche *et al.* in *J. Med. Chem., 43*:2946-2961 (2000).



xxxiv xxxv xxxvi

For some embodiments of the present invention, it is desirable to prepare compounds wherein R_2 is R''_2 as individual enantiomers. These may be obtained by methods described herein, and the individual enantiomers may be separated by methods such as crystallization or chiral chromatography as is known in the art. However, it is also possible to synthesize the individual enantiomers by chiral synthetic methods, using Schöllkopt methodology, for example. Both enantiomers may be prepared using this synthetic route and proper selection of the chiral auxiliary group. By sequential alkylation of the chiral template xxxvii, compound xxxviii is produced diastereoselectively. The chiral intermediate xli may be obtained therefrom by subsequent transformations including hydrolysis, reduction and protection.



xxxvii xxxviii xxxix

xl xli

Insofar as the production of the starting materials is not particularly described, the compounds are known or may be prepared analogously to methods known in the art or as disclosed in the Examples hereinafter.

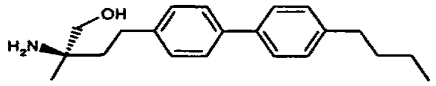
The following Examples are illustrative of the invention.

RT = room temperature

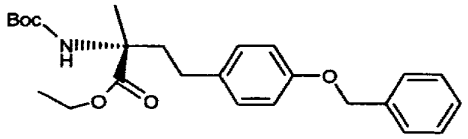
DCM = dichloromethane

Bn = benzyl

Example 1: (R)-2-Amino-4-(4'-butyl-biphenyl-4-yl)-2-methyl-butan-1-ol

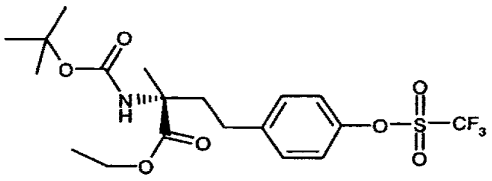


a) (R)-4-(4-Benzyloxy-phenyl)-2-tert-butoxycarbonylamino-2-methyl-butyric acid ethyl ester



To a solution of (2R,5R)-2-[2-(4-benzyloxy-phenyl)-ethyl]-3,6-diethoxy-5-isopropyl-2-methyl-2,5-dihydro-pyrazine (6.9 g, prepared as disclosed in WO 02/76695 the contents of which being herein incorporated by reference) in dry dioxane (170 ml) is added 105 ml of 0.5N HCl in water. After the homogenous solution is left standing overnight, ethyl acetate (300 ml) is added and the mixture is extracted with water (3 x 150 ml). The organic phase is dried ($MgSO_4$) and the solvent is evaporated. The crude product is dissolved in DCM and after addition of t-butyloxycarbonylanhydride (5.17 g) is left standing overnight. The solvent is removed in vacuo and the crude residue is purified by chromatography using diethyl ether/hexane (1/5) (R_f = 0.2, MS: (ES+): 428.5 $(M+H)^+$).

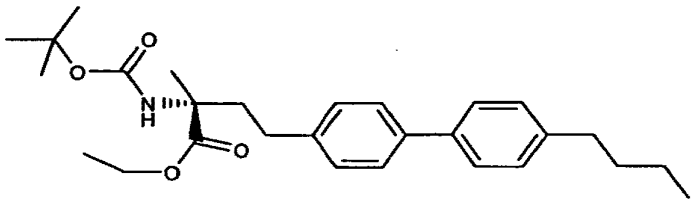
b) (R)-2-tert-Butoxycarbonylamino-2-methyl-4-(4-trifluoromethanesulfonyloxy-phenyl)-butyric acid ethyl ester



(R)-4-(4-Benzyloxy-phenyl)-2-tert-butoxycarbonylamino-2-methyl-butyric acid ethyl ester (2.78 g) is dissolved in ethyl acetate (100 ml) and hydrogenated at atmospheric pressure and RT using Pd/C (500 mg) for 16h. Filtration over talcum is followed by removal of the solvent in vacuo to yield a colorless oil (R_f (diethyl ether/hexane = 1/1) = 0.32, MS: (ES+): 338.4 $(M+H)^+$). The crude phenol (2.20g) and pyridine (2.6 ml) are dissolved in DCM and cooled to 0°C. Trifluoromethane sulfonic anhydride (1.3 ml) is added dropwise and the mixture is stirred at 0°C for 30 min. After addition of water (20 ml) and DCM (30 ml), the mixture is washed with 0.5N NaOH (15 ml), water (20 ml), 1M citric acid (2 x 25 ml) and

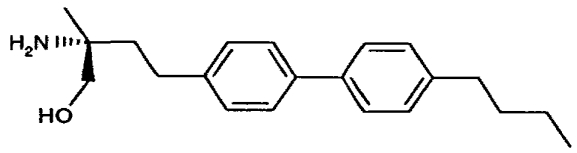
water (20 ml). The organic phase is dried over $MgSO_4$, the solvent removed and the crude material purified by chromatography using diethyl ether/hexane (1/2) giving the desired product as colorless oil (R_f = 0.44, MS: (ES+): 470.5 $(M+H)^+$).

c) (R)-2-tert-Butoxycarbonylamino-4-(4'-butyl-biphenyl-4-yl)-2-methyl-butyric acid ethyl ester



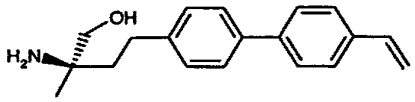
(R)-2-tert-Butoxycarbonylamino-2-methyl-4-(4-trifluoromethane sulfonyloxy-phenyl)-butyric acid ethyl ester (100 mg), 4-butylboronic acid (75 mg) and K_2CO_3 (44 mg) are suspended in dry toluene (3 ml). Argon is bubbled through the mixture for 10 min., tetrakispalladiumtriphenyl-phosphine (5mg) is added and the mixture is stirred at 95°C under Argon for 16h. After cooling to RT, ethyl acetate (5 ml) is added and the mixture is washed with 0.5N NaOH (2 ml), water (2 ml), 1M citric acid (2 x 2 ml) and water (2 ml). The organic phase is dried over $MgSO_4$, the solvent removed and the crude material purified by chromatography using diethylether/hexane = 1/5 (R_f = 0.14, MS: (ES+): 454.6 $(M+H)^+$).

d) (R)-2-Amino-4-(4'-butyl-biphenyl-4-yl)-2-methyl-butan-1-ol



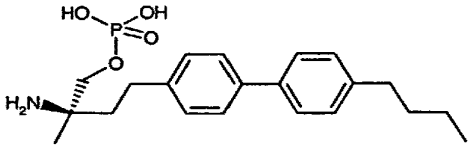
To a solution of (R)-2-tert-butoxycarbonylamino-4-(4'-butyl-biphenyl-4-yl)-2-methyl-butyric acid ethyl ester (22 mg) in diethylether is added lithium borohydride (20 mg). After stirring the suspension for 9h at RT, ethyl acetate (5 ml) is added and the mixture is washed with water (2 ml), 1M citric acid (2 x 2 ml) and water (2 ml). The organic phase is dried over $MgSO_4$, the solvent removed and the crude material purified by chromatography using diethyl ether/hexane (1/1) (R_f = 0.31, MS: (ES+): 412.6 $(M+H)^+$). The purified product is dissolved in dioxane containing 4M HCl and left at room temperature for 16h. After lyophilisation, the desired compound is obtained as a white solid in the hydrochloride salt form (R_f = 0.48 in DCM/methanol 100/15, MS: (ES+): 312.5 $(M+H)^+$).

Example 2: (R)-2-Amino-4-(4'-vinyl-biphenyl-4-yl)-2-methyl-butan-1-ol



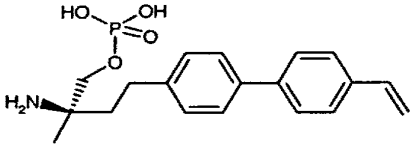
The title compound is obtained by following the procedure as disclosed in Example 1, but using the appropriate starting materials, e.g. vinylphenylboronic acid instead of 4-butylboronic acid in step c). The compound is obtained as an off-white solid, in the hydrochloride salt form. MS: (ES+): 282.4 $(M+H)^+$

Example 3: Phosphoric acid mono-{(R)-2-amino-4-(4'-butyl-biphenyl-4-yl)-2-methyl butyl} ester



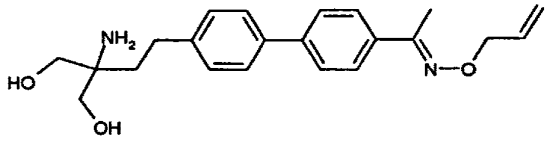
The compound of Example 1c) is converted into the corresponding phosphoric acid monoester by following a procedure as disclosed in WO 02/18395.

Example 4: Phosphoric acid mono-{(R)-2-amino-4-(4'-vinyl-biphenyl-4-yl)-2-methyl-butyl} ester



(R)-2-tert-Butoxycarbonylamino-4-(4'-vinyl-biphenyl-4-yl)-2-methyl-butyric acid ethyl ester is converted into the corresponding phosphoric acid monoester by following a procedure as disclosed in WO 02/18395.

Example 5: 1-[4'-(3-Amino-4-hydroxy-3-hydroxymethyl-butyl)-biphenyl-4yl]-ethanone-*O*-allyl-oxime



Step A: 2-Acetylamino-2-(2-biphenyl-4-yl-2-oxo-ethyl)-malonic acid diethyl ester

Sodium hydride (15 mmol) is added to anhydrous ethanol (50 mL). To this resulting sodium ethoxide solution is added 2-acetylaminomalonic acid diethyl ester (15 mmol) in one portion. The resulting mixture is stirred at room temperature for 30 min. A solution of 4'-phenyl-2-bromoacetophenone (10 mmol) in ethanol (10 mL) is then added and the resulting mixture is stirred at room temperature for 12 h. After concentrating under reduced pressure, the residue is dissolved in EtOAc and water. The organic phase is washed with brine and dried over Na_2SO_4. After removal of the solvent, the crude material is purified by column chromatography using EtOAc/hexane (1/3) giving the desired product as white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.6 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 7.45 (m, 3H), 7.13 (s, 1H), 4.28 (m, 6H), 1.98 (s, 2H), 1.26 (t, J = 7.1 Hz, 6H); MS: (ES^+): 412.2 $(M+1)^+$.

Step B: Acetic acid 4-acetoxy-2-acetoxymethyl-2-acetylamino-4-biphenyl-4-yl-butyl ester

To a solution of 2-acetylamino-2-(2-biphenyl-4-yl-2-oxo-ethyl)-malonic acid diethyl ester (5 mmol) in 95% EtOH (50 mL) is added $NaBH_4$ (25 mmol) in portions. After stirring at room temperature for 3 h, the reaction is quenched with saturated NH_4Cl. After removal of EtOH under reduced pressure, the aqueous solution is extracted with EtOAc. The organic phase is washed with brine and dried over Na_2SO_4. After concentrating, the residue is dissolved in anhydrous CH_2Cl_2 (25 mL). Ac_2O (30 mmol) and pyridine (60 mmol) are then added. After stirring at room temperature for 12 h, it is sequentially washed with 1 N HCl, saturated $NaHCO_3$, and brine and dried over Na_2SO_4. After removal of the solvent, the crude material is purified by column chromatography using EtOAc/hexane (1/1) to give desired product as a white solid. MS: (ES^+): 456.2 $(M+1)^+$.

Step C: Acetic acid 2-acetoxymethyl-2-acetylamino-4-biphenyl-4-yl-butyl ester

Acetic acid 4-acetoxy-2-acetoxymethyl-2-acetylamino-4-biphenyl-4-yl-butyl ester (5 mmol) is dissolved in EtOH (50 mL) and hydrogenated at atmospheric pressure using 10% Pd-C (10 %) at room temperature for 12 h. After filtration and concentration, the crude product is obtained as a white solid and used in the next step without further purification. MS: (ES^+): 398.2 $(M+1)^+$.

Step D: Acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-acetylbiphenyl-4-yl)-butyl ester

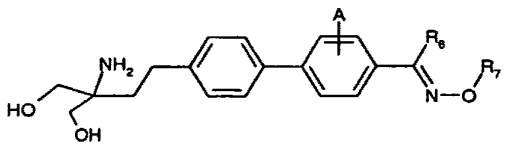
To a suspension of $AlCl_3$ (16 mmol) in DCE (20 mL) is added AcCl (8 mmol) in one portion. After stirring at room temperature for 30 min, to the solution is added acetic acid 2-acetoxymethyl-2-acetylamino-4-biphenyl-4-yl-butyl ester (2 mmol) in DCE (5 mL). After an additional 30 min, the mixture is poured into ice-cold 1 N NaOH and is extracted with DCM. The organic phase is washed with 1 N HCl, brine and dried over Na_2SO_4. After concentrating, the crude material is purified by column chromatography using EtOAc/hexane (2/1) to give the desired product as a white solid. MS: (ES^+): 439.2 $(M+1)^+$.

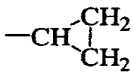
Step E: 1-[4'-(3-Amino-4-hydroxy-3-hydroxymethyl-butyl)-biphenyl-4-yl]ethanone-*O*-allyl-oxime

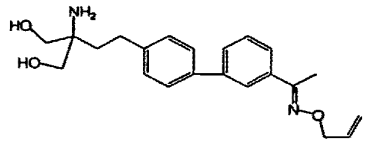
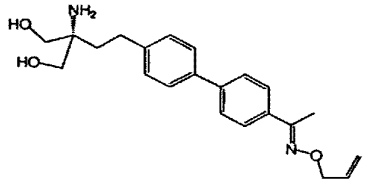
To a solution of 1-[4'-(3-amino-4-hydroxy-3-hydroxymethyl-butyl)-biphenyl-4-yl]ethanone-*O*-allyl-oxime (0.2 mmol) in MeOH (1 mL) is added *O*-allylalkoxylamine hydrochloride salt (0.24 mmol) and Et_3N (0.23 mmol). After stirring at room temperature for 12 h, it is concentrated and the residue is dissolved in DCM, which is washed with brine and dried over Na_2SO_4. After the concentration, the crude product is dissolved in THF (1 mL) and treated with 2 N LiOH aqueous solution (0.5 mL). The resulting mixture is stirred at reflux for 1 h and diluted with H_2O (10 mL). It is then extracted with EtOAc (3 x 5 mL) and the combined organic phase is washed with brine and dried over Na_2SO_4. After concentrating, the crude product is purified with LC-MS to give the desired product as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.6 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 7.45 (m, 3H), 7.13 (s, 1H), 4.28 (m, 6H), 1.98 (s, 2H), 1.26 (t, J = 7.1 Hz, 6H); MS: (ES^+): 369.2 $(M+1)^+$.

By repeating the procedure described in Example 5, using appropriate starting materials, the following compounds of Formula I are obtained as identified in Table I.

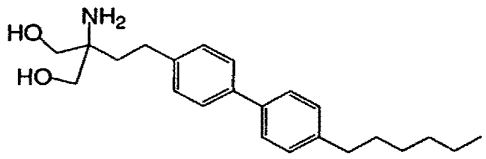
TABLE I

Example No.				Physical Data
	A	R_6	R_7	MS (M+1)

6	H	$-(CH_2)_2CH_3$	$-CH_3$	371.2
7	H	$-CH_3$	-H	329.2
8	H	$-CH_3$	$-CH_3$	343.2
9	H	$-CH_2CH_3$	$-CH_3$	357.2
10	H	$-(CH_2)_3CH_3$	$-CH_3$	385.2
11	H	$-(CH_2)_2CH_3$	$-CH_2CH_3$	385.2
12	H	$-(CH_2)_2CH_3$	$-CH_2CH=CH_2$	397.2
13	H	$-CH_2CH_3$	-H	343.2
14	H	$-CH_2CH_3$	$-CH_2CH_3$	371.2
15	H	$-CH_2CH_3$	$-CH_2CH=CH_2$	383.2
16	H		$-CH_2CH=CH_2$	395.3
17	H	$-(CH_2)_3CH_3$	$-CH_2CH=CH_2$	411.3
18	H	$-(CH_2)_3CH_3$	$-CH_2CH_3$	399.3
19	H	$-(CH_2)_4CH_3$	$-CH_2CH=CH_2$	425.3
20	H	$-(CH_2)_4CH_3$	$-CH_2CH_3$	413.3
21	H	$-(CH_2)_6CH_3$	$-CH_2CH=CH_2$	453.3
22	$-CH_3$ (*meta*)	$-CH_3$	$-CH_2CH=CH_2$	383.2
23	H	H	$-CH_2CH=CH_2$	355.2
24	H	$-CH_3$	$-(CH_2)_3CH_3$	385.2
25	H	$-CH_3$	$-(CH_2)_2CH_3$	371.2
26	H	$-CH_3$	$-(CH_2)_4CH_3$	399.2
27	H	$-CH_3$	$-(CH_2)_5CH_3$	413.2
28	I (*meta*)	$-CH_3$	$-(CH_2)_2CH_3$	
28.1	F (*meta*)	$-CH_3$	$-(CH_2)_2CH_3$	389.2

29	I (*meta*)	$-CH_3$	$-CH_2CH=CH_2$	
29.1	F (*meta*)	$-CH_3$	$-CH_2CH=CH_2$	387.2
30	I (*ortho*)	$-CH_3$	$-CH_2CH=CH_2$	
30.1	F (*ortho*)	$-CH_3$	$-CH_2CH=CH_2$	387.2
31	I (*ortho*)	$-CH_3$	$-(CH_2)_2CH_3$	
31.1	F (*ortho*)	$-CH_3$	$-(CH_2)_2CH_3$	389.2
32	H	$-CH_3$	$-CH_2C\equiv CH$	367.2
33				369.2
34				369.2

Example 35: 2-Amino-2-[2-(4'-hexylbiphenyl-4-yl)-ethyl]propane-1,3-diol



Step A: Acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-hexyl-biphenyl-4-yl)-butyl ester

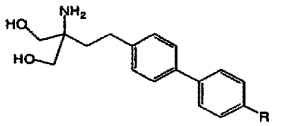
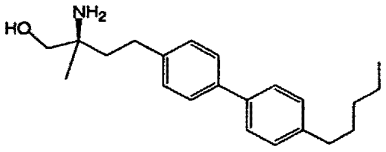
To a solution of acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-hexanoyl-biphenyl-4-yl)-butyl ester (prepared according to Scheme 1) (1 mmol) in trifluoroacetic acid (10 mL) is added triethylsilane (2.5 mmol). The resulting mixture is stirred at room temperature for 12 h. After concentrating under reduced pressure, the residue is dissolved in DCM and the organic solution is washed with saturated $NaHCO_3$ and brine and dried over Na_2SO_4. After concentration, the crude product is purified by column chromatography using EtOAc/hexane (1/1) to give the desired compound as a white solid. MS: (ES^+): 482.3 $(M+1)^+$.

Step B: 2-Amino-2-[2-(4'-hexylbiphenyl-4-yl)-ethyl]propane-1,3-diol

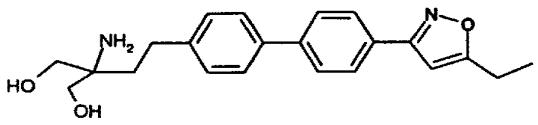
Acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-hexyl-biphenyl-4-yl)-butyl ester (0.2 mmol) is dissolved in THF (1 mL) and treated with 2 N LiOH aqueous solution (0.5 mL). The resulting mixture is stirred at reflux for 1 h and diluted with H_2O (10 mL). It is then extracted with EtOAc (3 x 5 mL) and the combined organic phase is washed with brine and dried over Na_2SO_4. After concentrating, the crude product is purified with LC-MS to give the desired product as a white solid. MS: (ES^+): 356.2 $(M+1)^+$.

By repeating the procedure described in Example 35, using appropriate starting materials, the following compounds of Formula I are obtained as identified in Table II.

TABLE II

Example No.	 R	Physical Data MS (M+1)
36	$-(CH_2)_4CH_3$	342.2
37	$-(CH_2)_7CH_3$	384.3
38	$-C(=CHCH_2CH_3)(CH_2)_2CH_3$	368.3
39		326.2

Example 40: 2-Amino-2-{2-[4'-(5-propyl-isoxazol-3-yl)-biphenyl-4-yl]-ethyl}-propane-1,3-diol



Step A: 4-[2-(4-Bromophenyl)vinyl]-4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazole

To a suspension of (4-bromobenzyl)triphenyl-phosphonium bromide (6 mmol) in dry THF (25 mL) is added NaH (6 mmol) in portions. After stirring at room temperature for 30 min, a solution of 4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazole-4-carbaldehyde (prepared according to Scheme 3 using well-known chemistry in the art) (5 mmol) in THF (10 mL) is added in one portion. The mixture is stirred at room temperature for 12 h. After concentration, the residue is treated with EtOAc/hexane (1/5) (100 mL) and the solid is filtrated. The filtrate is washed with brine and dried over Na_2SO_4. After concentration, the crude product is purified by column chromatography by EtOAc/hexane (1/5) to give the desired product as a colorless oil. MS: (ES^+): 410.1 $(M+1)^+$.

Step B: 4-[2-(4-Bromophenyl)ethyl]-4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazole

4-[2-(4-Bromophenyl)vinyl]-4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazole (3 mmol) is dissolved in ethanol (15 mL) and hydrogenated at atomospheric pressure in the presence of chlorotris(triphenylphosphine)rhodium(I) (10 %). The mixture is stirred at 40 ^{0}C for 12 h. After filtration and concentration, the crude product is obtained as colorless oil, which is used directly in the next step without further purification. MS: (ES^+): 412.1 $(M+1)^+$.

a) Step C: 4-{2-[4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazol-4-yl]ethyl}-biphenyl-4-carbaldehyde

The mixture of 4-[2-(4-bromophenyl)ethyl]-4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazole (2 mmol), 4-fomylphenylboronic acid (2.4 mmol), $Pd(PPh_3)_4$ (0.2 mmol) and Na_2CO_3 (9.6 mmol) in toluene (5 mL), EtOH (1.5 mL) and H_2O (5 mL) is stirred at 90°C for 5 h. It is diluted with H_2O (15 mL) and EtOAc (15 mL) and the organic phase is washed with brine and dried over Na_2SO_4. After concentration, the crude product is purified by column chromatography using EtOAc/hexane (1/4) to give the desired product as a white solid. MS: (ES^+): 438.2 $(M+1)^+$.

b) Step D: Acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-formylbiphenyl-4-yl)butyl ester

To a solution of 4-{2-[4-(*t*-Butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazol-4-yl]ethyl}-biphenyl-4-carbaldehyde (2 mmol) in THF (10 mL) is added 1 N HCl aqueous solution (5 mL). The mixture is refluxed for 2 hours. After cooling to room temperature, it is

neutralized by saturated Na_2CO_3 and extracted with EtOAc (20 X 3). The combined organic phase is washed with brine and dried over Na_2SO_4. After concentrating, the residue is dissolved in dry DCM (10 mL) and is treated with Ac_2O (8 mmol) and pyridine (16 mmol). After stirring at room temperature for 12 h, the solution is washed with 1 N HCl and brine and dried over Na_2SO_4. After concentrating, the crude product is purified by column chromatography using EtOAc/hexane (1/1) to give the desired product as white solid. MS: (ES^+): 426.2 $(M+1)^+$.

c) Step E: Acetic acid 2-acetoxymethyl-2-acetylamino-4-[4'-(hydroxyiminomethyl)-biphenyl-4-yl]butyl ester

To a solution of acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-formyl-biphenyl-4-yl) butyl ester (1 mmol) in methanol (10 mL) is added $NH_2OH.HCl$ (1.2 mmol) and Et_3N (1.1 mmol). The mixture is stirred at room temperature 12 hours. After concentrating, the residue is dissolved in DCM (20 mL) and washed with H_2O and brine. The crude product, after concentration, is used in the next step without further purification. MS: (ES^+): 441.2 $(M+1)^+$.

d) Step F: Acetic acid 2-acetoxymethyl-2-acetylamino-4-[4'-(5-propyl-isoxazol-3-yl)biphenyl-4-yl]butyl ester

A mixture of acetic acid 2-acetoxymethyl-2-acetylamino-4-[4'-(hydroxyimino-methyl)biphenyl-4-yl]butyl ester (0.2 mmol), NaOCl (2 mmol), Et_3N (3 mmol) and pentyne (40 mmol) in DCM (4 mL) and H_2O (1 mL) is stirred at room temperature for 12 h. It is diluted with DCM (5 mL) and H_2O (10 mL) and the organic phase is washed with brine and dried over Na_2SO_4. After concentrating, the crude product is purified by column chromatography using EtOAc/hexane (1/1) to give the desired product as a white solid. MS: (ES^+): 507.2 $(M+1)^+$.

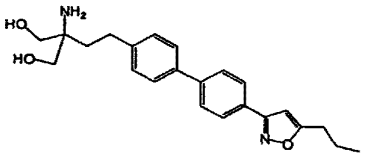
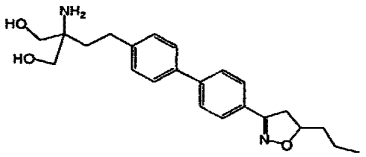
e) Step G: 2-Amino-2-{2-[4'-(5-propyl-isoxazol-3-yl)-biphenyl-4-yl]ethyl}propane-1,3-diol

Acetic acid 2-acetoxymethyl-2-acetylamino-4-[4'-(5-propyl-isoxazol-3-yl)-biphenyl-4-yl]butyl ester (0.1 mmol) is dissolved in THF (1 mL) and treated with 2 N LiOH aqueous solution (0.5 mL). The resulting mixture is stirred at reflux for 1 h and diluted with H_2O (10 mL). It is then extracted with EtOAc (3 x 5 mL) and the combined organic phase is washed with brine and dried over Na_2SO_4. After concentrated, the crude product is purified with LC-MS to give the desired product as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 7.86 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 3.53 (q, J = 11.0

Hz, 4H), 2.81 (t, J = 7.4 Hz, 2H), 2.71 (m, 2H), 1.79 (m, 4H), 1.04 (t, J = 7.4 Hz, 3H). MS: (ES$^+$): 381.2 $(M+1)^+$.

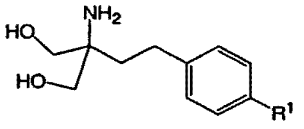
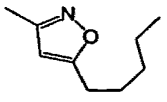
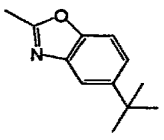
By repeating the procedure described in Example 40, using appropriate starting materials, the following compounds of formula I are obtained as identified in Table III.

TABLE III

Example No.	Structure	Physical Data MS (M+1)
41		381.2
42		383.2

By repeating the appropriate procedure described above, using appropriate starting materials, the following compounds of formula I are obtained as identified in Tables IV, V and VI.

TABLE IV

Example No.	 R^1	Physical Data MS (M+1)
43		333.2
44		369.2

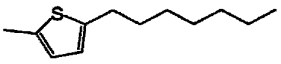
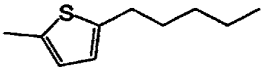
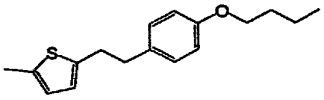
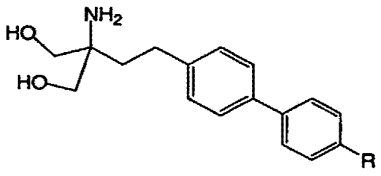
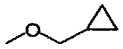
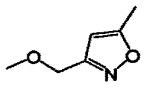
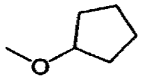
45		376.2
46		348.2
47		454.2

TABLE V

Example No.	 R	Physical Data MS (M+1)
48	-O(CH$_2$)$_2$CH$_3$	330.2
49		342.2
50	-O(CH$_2$)$_3$CH$_3$	344.2
51	-OCH$_2$CH$_3$	316.2
52	-O(CH$_2$)$_2$CH(CH$_3$)$_2$	358.2
53	-O(C$_6$H$_5$)	364.2
54	-O(CH$_2$)$_4$CH$_3$	358.2
55		383.2
56	-O(CH$_2$)$_2$(C$_6$H$_5$)	392.2
57		356.2
58	-O(CH$_2$)$_2$OCH$_2$CH$_3$	360.2
59	-O(CH$_2$)$_2$OCH$_3$	346.2

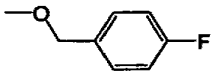
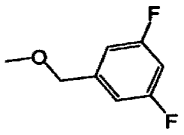
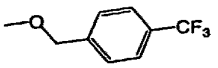
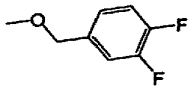
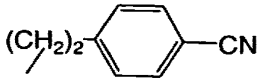
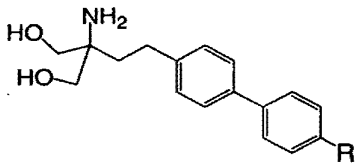
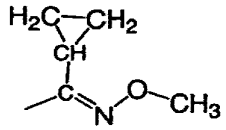
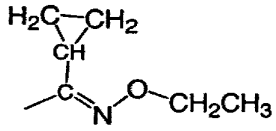
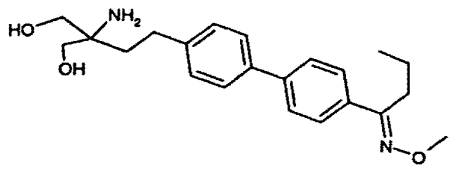
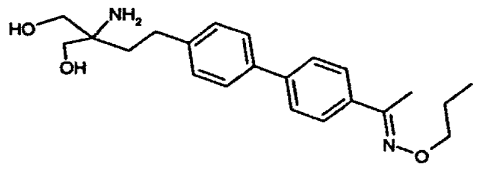
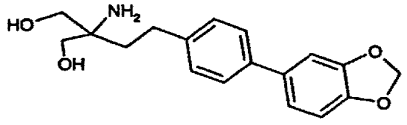
60		396.2
61		414.2
62		446.2
63		414.2
64		401.2

TABLE VI

Example No.	 R	Physical Data MS (M+1)
65	H	272.2
66	-C(O)$(CH_2)_2CH_3$	342.2
67	-CH(OH)$(CH_2)_2CH_3$	344.2
68	-C(O)CH_2CH_3	328.2
69	-C(O)$(CH_2)_3CH_3$	356.2
70	-$(CH_2)_3CH_3$	328.2
71	-CH(OH)CH_2CH_3	330.2
72	-CH(OH)$(CH_2)_3CH_3$	358.2
73	-$(CH_2)_2CH_3$	314.2
74	-C(=NOH)$(CH_2)_2CH_3$	357.2

75	-C(=NOH)$(CH_2)_3CH_3$	371.2
76	-C(=NOCH_2CH_3)CH_3	357.2
77		369.2
78		383.2
79	-C(=NOCH_2CH_3)$(CH_2)_6CH_3$	441.3
80	-CH(CH_2CH_3)$(CH_2)_2CH_3$	356.3
81	-CH($(CH_2)_2CH_3)_2$	370.3
82	-NHS$(O)_2CH_3$	365.2
83	-NH_2	287.2
84	-C(O)NH$(CH_2)_3CH_3$	371.2
85	-CH_2CN	311.2
86	-OCH_2CN	327.2
87	-OCH_2C≡CH	326.2
88	-OH	288.2
89	-O$(CH_2)_3$F	348.2
90	-O$(CH_2)_7CH_3$	400.3
91	-O$(CH_2)_6CH_3$	386.3
92		371.2

93		371.2
94		316.2

The compounds of formula I in free form or in pharmaceutically acceptable salt form, exhibit valuable pharmacological properties, e.g. lymphocyte recirculation modulating or anti-angiogenic properties, e.g. as indicated in in vitro and in vivo tests and are therefore indicated for therapy.

A. In vitro: Binding affinity of compounds of formula I to individual human S1P receptors may be determined in the following assays:

Transient transfection of human S1P receptors into HEK293 cells

EDG receptors and G_i proteins are cloned, and equal amounts of 4 cDNAs for the EDG receptor, G_i-α, G_i-β and G_i-γ are mixed and used to transfect monolayers of HEK293 cells using the calcium phosphate precipitate method (M. Wigler et al., Cell. 1977;11;223 and DS. Im et al., Mol. Pharmacol. 2000;57;753). Briefly, a DNA mixture containing 25 μg of DNA and 0.25 M CaCl is added to HEPES-buffered 2 mM Na_2HPO_4. Subconfluent monolayers of HEK293 cells are poisoned with 25 mM chloroquine, and the DNA precipitate is then applied to the cells. After 4 hours, the monolayers are washed with phosphate-buffered saline and re-fed media (90% 1:1 Dulbecco's modified essential media (DMEM):F-12 + 10% fetal bovine serum). The cells are harvested 48-72 hours after addition of the DNA by scraping in HME buffer (in mM: 20 HEPES, 5 $MgCl_2$, 1 EDTA, pH 7.4) containing 10% sucrose on ice, and disrupted using a Dounce homogenizer. After centrifugation at 800xg, the supernatant is diluted with HME without sucrose and centrifuged at 100,000xg for 1 hour. The resulting pellet is rehomogenized and centrifuged a second hour at 100,000xg. This crude membrane pellet is resuspended in HME with sucrose, aliquoted, and snap-frozen by immersion in liquid nitrogen. The membranes are stored at 70°C. Protein concentration is determined spectroscopically by the Bradford protein assay.

Example	**$S1P_1$** EC_{50} [nM]	**$S1P_2$** EC_{50} [nM]	**$S1P_3$** EC_{50} [nM]	**$S1P_4$** EC_{50} [nM]	**$S1P_5$** EC_{50} [nM]
36	0.33	>10000	>10000	1.2	1.1

41	0.16	>10000	53.8	>10000	2.1
63	0.07	>10000	1.9	>10000	0.1

GTPγS binding assay using S1P receptor/HEK293 membrane preparations

GTPγS binding experiments are performed as described by DS. Im et al., Mol. Pharmacol. 2000; 57:753. Ligand-mediated GTPγS binding to G-proteins is measured in GTP binding buffer (in mM: 50 HEPES, 100 NaCl, 10 $MgCl_2$, pH 7.5) using 25 μg of a membrane preparation from transiently transfected HEK293 cells. Ligand is added to membranes in the presence of 10 μM GDP and 0.1 nM [^{35}S]GTPγS (1200 Ci/mmol) and incubated at 30°C for 30 min. Bound GTPγS is separated from unbound using the Brandel harvester (Gaithersburg, MD) and counted with a liquid scintillation counter.

In these assays, the compounds of formula I wherein R_2 or R_5 is a residue of formula (h) have binding affinities to S1P receptors in the sub-microM range.

B. In Vitro: Antitumor Activity

A mouse breast cancer cell line originally isolated from mammary carcinomas is used, e.g. JygMC(A). The cell number is adjusted to $5x10^5$ for plating in fresh medium before the procedure. Cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12 hours and then washed twice with PBS, followed by addition of fresh medium with 10% FCS and additionally incubated for another 12 hours. Thereafter the cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12 hours. To release the cells from the block, the cells are washed twice with PBS and replated in fresh medium with 10% FCS. After synchronisation, the cells are incubated with or without various concentrations of a compound of formula I for 3, 6, 9, 12, 18 or 24 hours. The cells are harvested after treatment with 0.2% EDTA, fixed with ice-cold 70% ethanol solution, hydrolyzed with 250μg/ml of RNaseA (type 1-A: Sigma Chem. Co.) at 37°C for 30 minutes and stained with propidium iodide at 10mg/ml for 20 minutes. After the incubation period, the number of cells is determined both by counting cells in a Coulter counter and by the SRB colorimetric assay. Under these conditions compounds of formula I inhibit the proliferation of the tumor cells at concentrations ranging from 10^{-12} to 10^{-6} M.

C. In vitro: S1P-Mediated HUVEC Migration Assay

The migration assay is performed using Fluoro-Blok 24-Multiwell Insert Plates coated with fibronectin (8 μm pore size, Falcon #351147) instead of the individual inserts in a 24-well

plate. Cells and test compounds are prepared and pre-incubated as described above, then 100 µl is added to each approriate well in the Insert Plate. 300 µl of the EBM-2 + 2 % charcoal-stripped media without S1P is added to the bottoms of the wells marked for no stimulation (-), and 300 µl of the media containing S1P (500 nM) is added to the bottoms of the wells marked for stimulation (+). The plate is then incubated for 4 hours at 37 ºC, 5 % CO_2.

Calcein AM, 50 µg/vial, (Molecular Probes #C3100) is prepared by first adding 20 µl DMSO to the vial. Then 12.5 ml of HBSS (per plate) is warmed to 37ºC and 150 µl is added to the vial. The contents of the vial are then transferred back to the remaining HBSS to make the final concentration 4 µg/ml Calcein AM.

The Fluoro-Blok plate is removed from the incubator and the top insert plate is separated and "flicked" to remove excess media clinging to the inserts. The insert plate is then transferred to a fresh 24-well plate containing 500 µl/well of the 4 µg/ml Calcein AM. The plate is then incubated for 1.5 hours at 37 ºC, 5 % CO_2.

After incubation, the plate is read on a Cytofluor II at an excitation of 485 nm and emission of 530 nm. The Fluoro-Blok coating in the inserts allows only the cells that have migrated to the bottom to be counted. Data are transferred to Excel for calculations, graphs are created using SigmaPlot, and SigmaStat is used for significance tests (t-test).

D. In vivo: Blood Lymphocyte Depletion

A compound of formula I or the vehicle is administered orally by gavage to rats. Tail blood for hematological monitoring is obtained on day –1 to give the baseline individual values, and at 2, 6, 24, 48 and 72 hours after drug application. In this assay, the compounds of formula I deplete peripheral blood lymphocytes when administered at a dose of 0.03 to 3 mg/kg. For example compounds of Example 2 and 9 deplete peripheral blood lymphocytes by more than 50% 6 hours after administration of a dose of 0.8 mg/kg and 0.2 mg/kg, respectively.

E. In vivo: Screening Assays for measurement of circulating lymphocytes and assessment of heart effect

Measurement of Circulating Lymphocytes: Compounds are dissolved in DMSO and further diluted with deionized water. Mice (C57bl/6 male, 6-10 week-old) are administered 20 μg of compounds (diluted in 200 μl water, 4% DMSO) via intra-peritoneal (IP) injection under short isoflurane anesthesia. 200 μl water, 4% DMSO, and FTY720 (10 μg) are included as negative and positive controls.

Blood is collected from the retro-orbital sinus 18 hours after drug administration under short isoflurane anesthesia. Whole blood samples are subjected to hematology analysis. Peripheral lymphocyte counts are determined using an automated analyzer (Hemavet 3700). Subpopulations of peripheral blood lymphocytes are stained by fluorochrome-conjugated specific antibodies and analyzed using a fluorescent activating cell sorter (Facscalibur). Two mice are used to assess the lymphocyte depletion activity of each compound screened.

Assessment of Heart Effect: The effects of compounds on cardiac function are monitored using the AnonyMOUSE ECG recording system. ECGs are recorded in conscious mice (C57bl/6 male, 6-10 week-old) before and after compound administration. 90 μg of compound further diluted in 200 μl water and 15% DMSO are injected IP. Four mice are used to assess heart rate effect of each compound.

F. In vivo: Anti-angiogenic Activity

Porous chambers containing (i) sphingosine-1-phosphate (5 μM/chamber) or (ii) human VEGF (1 μg/chamber) in 0.5 ml of 0.8% w/v agar (containing heparin, 20 U/ml) are implanted subcutaneously in the flank of mice. S1P or VEGF induces the growth of vascularized tissue around the chamber. This response is dose-dependent and can be quantified by measuring the weight and blood content of the tissue. Mice are treated once a day orally or intravenously with a compound of formula I starting 4-6 hours before implantation of the chambers and continuing for 4 days. The animals are sacrificed for measurement of the vascularized tissues 24 hours after the last dose. The weight and blood content of the vascularized tissues around the chamber is determined. Animals treated with a compound of formula I show reduced weight and/or blood content of the vascularized tissues compared to animals treated with vehicle alone. Compounds of formula I are anti-angiogenic when administered at a dose of about 0.3 to about 3 mg/kg.

The compounds of formula I are, therefore, useful in the treatment and/or prevention of diseases or disorders mediated by lymphocytes interactions, e.g. in transplantation, such as acute or chronic rejection of cell, tissue or organ allo- or xenografts or delayed graft function,

graft versus host disease, autoimmune diseases, e.g. rheumatoid arthritis, systemic lupus erythematosus, hashimoto's thyroidis, multiple sclerosis, myasthenia gravis, diabetes type I or II and the disorders associated therewith, vasculitis, pernicious anemia, Sjoegren syndrome, uveitis, psoriasis, Graves ophthalmopathy, alopecia areata and others, allergic diseases, e.g. allergic asthma, atopic dermatitis, allergic rhinitis/conjunctivitis, allergic contact dermatitis, inflammatory diseases optionally with underlying aberrant reactions, e.g. inflammatory bowel disease, Crohn's disease or ulcerative colitis, intrinsic asthma, inflammatory lung injury, inflammatory liver injury, inflammatory glomerular injury, atherosclerosis, osteoarthritis, irritant contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, cutaneous manifestations of immunologically-mediated disorders, inflammatory eye disease, keratoconjunctivitis, myocarditis or hepatitis, ischemia/reperfusion injury, e.g. myocardial infarction, stroke, gut ischemia, renal failure or hemorrhage shock, traumatic shock, T cell lymphomas or T cell leukemias, infectious diseases, e.g. toxic shock (e.g. superantigen induced), septic shock, adult respiratory distress syndrome or viral infections, e.g. AIDS, viral hepatitis, chronic bacterial infection, or senile dementia. Examples of cell, tissue or solid organ transplants include e.g. pancreatic islets, stem cells, bone marrow, corneal tissue, neuronal tissue, heart, lung, combined heart-lung, kidney, liver, bowel, pancreas, trachea or oesophagus. For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired.

Furthermore, the compounds of formula I are useful in cancer chemotherapy, particularly for cancer chemotherapy of solid tumors, e.g. breast cancer, or as an anti-angiogenic agent.

In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.03 to 2.5 mg/kg per body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5 mg to about 100 mg, conveniently administered, e.g. in divided doses up to four times a day or in retard form. Suitable unit dosage forms for oral administration comprise from ca. 1 to 50 mg active ingredient.

The compounds of formula I may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, or parenterally, e.g. in the form of injectable solutions or suspensions, topically, e.g. in the form of lotions, gels, ointments or creams, or in a nasal or a suppository form. Pharmaceutical compositions comprising a compound of formula I in free form or in pharmaceutically acceptable salt form in association

with at least one pharmaceutical acceptable carrier or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent.

The compounds of formula I may be administered in free form or in pharmaceutically acceptable salt form e.g. as indicated above. Such salts may be prepared in a conventional manner and exhibit the same order of activity as the free compounds.

In accordance with the foregoing the present invention further provides:

1.1 A method for preventing or treating disorders or diseases mediated by lymphocytes, e.g. such as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

1.2 A method for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases, e.g. as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

1.3 A method for inhibiting or controlling deregulated angiogenesis, e.g. sphingosine-1-phosphate (S1P) mediated angiogenesis, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

1.4 A method for preventing or treating diseases mediated by a neo-angiogenesis process or associated with deregulated angiogenesis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

2. A compound of formula I, in free form or in a pharmaceutically acceptable salt form for use as a pharmaceutical, e.g. in any of the methods as indicated under 1.1 to 1.4 above.

3. A pharmaceutical composition, e.g. for use in any of the methods as in 1.1 to 1.4 above comprising a compound of formula I in free form or pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent or carrier therefor.

4. A compound of formula I or a pharmaceutically acceptable salt thereof for use in the preparation of a pharmaceutical composition for use in any of the method as in 1.1 to 1.4 above.

The compounds of formula I may be administered as the sole active ingredient or in conjunction with, e.g. as an adjuvant to, other drugs e.g. immunosuppressive or immunomodulating agents or other anti-inflammatory agents, e.g. for the treatment or prevention of allo- or xenograft acute or chronic rejection or inflammatory or autoimmune disorders, or a chemotherapeutic agent, e.g. a malignant cell anti-proliferative agent. For example the compounds of formula I may be used in combination with a calcineurin inhibitor, e.g. cyclosporin A or FK 506; a mTOR inhibitor, e.g. rapamycin, 40-O-(2-hydroxyethyl)-rapamycin, CCI779, ABT578 or AP23573; an ascomycin having immunosuppressive properties, e.g. ABT-281, ASM981, etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; leflunomide; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine or an immunosuppressive homologue, analogue or derivative thereof; immunosuppressive monoclonal antibodies, e.g. monoclonal antibodies to leukocyte receptors, e.g. MHC, CD2, CD3, CD4, CD7, CD8, CD25, CD28, CD40. CD45, CD58, CD80, CD86 or their ligands; other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g. CTLA4Ig (for ex. designated ATCC 68629) or a mutant thereof, e.g. LEA29Y ; adhesion molecule inhibitors, e.g. LFA-1 antagonists, ICAM-1 or -3 antagonists, VCAM-4 antagonists or VLA-4 antagonists; or a chemotherapeutic agent.

By the term "chemotherapeutic agent" is meant any chemotherapeutic agent and it includes but is not limited to,

i. an aromatase inhibitor,
ii. an anti-estrogen, an anti-androgen (especially in the case of prostate cancer) or a gonadorelin agonist,
iii. a topoisomerase I inhibitor or a topoisomerase II inhibitor,
iv. a microtubule active agent, an alkylating agent, an antineoplastic antimetabolite or a platin compound,
v. a compound targeting/decreasing a protein or lipid kinase activity or a protein or lipid phosphatase activity, a further anti-angiogenic compound or a compound which induces cell differentiation processes,

vi. a bradykinin 1 receptor or an angiotensin II antagonist,

vii. a cyclooxygenase inhibitor, a bisphosphonate, a histone deacetylase inhibitor, a heparanase inhibitor (prevents heparan sulphate degradation), e.g. PI-88, a biological response modifier, preferably a lymphokine or interferons, e.g. interferon γ, an ubiquitination inhibitor, or an inhibitor which blocks anti-apoptotic pathways,

viii. an inhibitor of Ras oncogenic isoforms, e.g. H-Ras, K-Ras or N-Ras, or a farnesyl transferase inhibitor, e.g. L-744,832 or DK8G557,

ix. a telomerase inhibitor, e.g. telomestatin,

x. a protease inhibitor, a matrix metalloproteinase inhibitor, a methionine aminopeptidase inhibitor, e.g. bengamide or a derivative thereof, or a proteosome inhibitor, e.g. PS-341, and/or

xi. a mTOR inhibitor.

The term "aromatase inhibitor" as used herein relates to a compound which inhibits the estrogen production, i.e. the conversion of the substrates androstenedione and testosterone to estrone and estradiol, respectively. The term includes, but is not limited to steroids, especially atamestane, exemestane and formestane and, in particular, non-steroids, especially aminoglutethimide, roglethimide, pyridoglutethimide, trilostane, testolactone, ketokonazole, vorozole, fadrozole, anastrozole and letrozole. A combination of the invention comprising a chemotherapeutic agent which is an aromatase inhibitor is particularly useful for the treatment of hormone receptor positive tumors, e.g. breast tumors.

The term "anti-estrogen" as used herein relates to a compound which antagonizes the effect of estrogens at the estrogen receptor level. The term includes, but is not limited to tamoxifen, fulvestrant, raloxifene and raloxifene hydrochloride. A combination of the invention comprising a chemotherapeutic agent which is an anti-estrogen is particularly useful for the treatment of estrogen receptor positive tumors, e.g. breast tumors.

The term "anti-androgen" as used herein relates to any substance which is capable of inhibiting the biological effects of androgenic hormones and includes, but is not limited to, bicalutamide.

The term "gonadorelin agonist" as used herein includes, but is not limited to abarelix, goserelin and goserelin acetate.

The term "topoisomerase I inhibitor" as used herein includes, but is not limited to topotecan, irinotecan, 9-nitrocamptothecin and the macromolecular camptothecin conjugate PNU-166148 (compound A1 in WO99/17804).

The term "topoisomerase II inhibitor" as used herein includes, but is not limited to the anthracyclines such as doxorubicin, daunorubicin, epirubicin, idarubicin and nemorubicin, the anthraquinones mitoxantrone and losoxantrone, and the podophillotoxines etoposide and teniposide.

The term "microtubule active agent" relates to microtubule stabilizing and microtubule destabilizing agents including, but not limited to taxanes, e.g. paclitaxel and docetaxel, vinca alkaloids, e.g., vinblastine, especially vinblastine sulfate, vincristine especially vincristine sulfate, and vinorelbine, discodermolides and epothilones and derivatives thereof, e.g. epothilone B or a derivative thereof.

The term "alkylating agent" as used herein includes, but is not limited to busulfan, chlorambucil, cyclophosphamide, ifosfamide, melphalan or nitrosourea (BCNU or Gliadel™).

The term "antineoplastic antimetabolite" includes, but is not limited to 5-fluorouracil, capecitabine, gemcitabine, cytarabine, fludarabine, thioguanine, methotrexate and edatrexate.

The term "platin compound" as used herein includes, but is not limited to carboplatin, cis-platin and oxaliplatin.

The term "compounds targeting/decreasing a protein or lipid kinase activity or further anti-angiogenic compounds" as used herein includes, but is not limited to protein tyrosine kinase and/or serine and/or threonine kinase inhibitors or lipid kinase inhibitors, e.g. compounds targeting, decreasing or inhibiting the activity of the epidermal growth factor family of receptor tyrosine kinases (EGFR, ErbB2, ErbB3, ErbB4 as homo- or heterodimers), the vascular endothelial growth factor family of receptor tyrosine kinases (VEGFR), the platelet-derived growth factor-receptors (PDGFR), the fibroblast growth factor-receptors (FGFR), the insulin-like growth factor receptor 1 (IGF-1R), the Trk receptor tyrosine kinase family, the Axl receptor tyrosine kinase family, the Ret receptor tyrosine kinase, the Kit/SCFR receptor tyrosine kinase, members of the c-Abl family and their gene-fusion products (e.g. BCR-Abl), members of the protein kinase C (PKC) and Raf family of serine/threonine kinases, members of the MEK, SRC, JAK, FAK, PDK or PI(3) kinase family, or of the PI(3)-kinase-related kinase family, and/or members of the cyclin-dependent kinase family (CDK) and anti-angiogenic compounds having another mechanism for their activity, e.g. unrelated to protein or lipid kinase inhibition.

Compounds which target, decrease or inhibit the activity of VEGFR are especially compounds, proteins or antibodies which inhibit the VEGF receptor tyrosine kinase, inhibit a VEGF receptor or bind to VEGF, and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 98/35958, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or a pharmaceutically acceptable salt thereof, e.g. the succinate, in WO 00/27820, e.g. a N-aryl(thio) anthranilic acid amide derivative e.g. 2-[(4-pyridyl)methyl]amino-N-[3-methoxy-5-(trifluoromethyl)phenyl]benzamide or 2-[(1-oxido-4-pyridyl)methyl]amino-N-[3-trifluoromethylphenyl]benzamide, or in WO 00/09495, WO 00/59509, WO 98/11223, WO 00/27819 and EP 0 769 947; those as described by M. Prewett et al in Cancer Research 59 (1999) 5209-5218, by F. Yuan et al in Proc. Natl. Acad. Sci. USA, vol. 93, pp. 14765-14770, Dec. 1996, by Z. Zhu et al in Cancer Res. 58, 1998, 3209-3214, and by J. Mordenti et al in Toxicologic Pathology, Vol. 27, no. 1, pp 14-21, 1999; in WO 00/37502 and WO 94/10202; Angiostatin™, described by M. S. O'Reilly et al, Cell 79, 1994, 315-328; Endostatin™, described by M. S. O'Reilly et al, Cell 88, 1997, 277-285; anthranilic acid amides; ZD4190; ZD6474; SU5416; SU6668; or anti-VEGF antibodies or anti-VEGF receptor antibodies,e.g. RhuMab.

By antibody is meant intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies formed from at least 2 intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

Compounds which target, decrease or inhibit the activity of the epidermal growth factor receptor family are especially compounds, proteins or antibodies which inhibit members of the EGF receptor tyrosine kinase family, e.g. EGF receptor, ErbB2, ErbB3 and ErbB4 or bind to EGF or EGF related ligands, or which have a dual inhibiting effect on the ErbB and VEGF receptor kinase and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 97/02266, e.g. the compound of ex. 39, or in EP 0 564 409, WO 99/03854, EP 0520722, EP 0 566 226, EP 0 787 722, EP 0 837 063, US 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and, especially, WO 96/30347 (e.g. compound known as CP 358774), WO 96/33980 (e.g. compound ZD 1839) and WO 95/03283 (e.g. compound ZM105180) or PCT/EP02/08780; e.g. trastuzumab (Herpetin®), cetuximab, Iressa, OSI-774, CI-1033, EKB-569, GW-2016, E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6.3 or E7.6.3.

Compounds which target, decrease or inhibit the activity of PDGFR are especially compounds which inhibit the PDGF receptor, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib.

Compounds which target, decrease or inhibit the activity of c-Abl family members and their gene fusion products are, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib; PD180970; AG957; or NSC 680410.

Compounds which target, decrease or inhibit the activity of protein kinase C, Raf, MEK, SRC, JAK, FAK and PDK family members, or PI(3) kinase or PI(3) kinase-related family members, and/or members of the cyclin-dependent kinase family (CDK) are especially those staurosporine derivatives disclosed in EP 0 296 110, e.g. midostaurin; examples of further compounds include e.g. UCN-01, safingol, BAY 43-9006, Bryostatin 1, Perifosine; Ilmofosine; RO 318220 and RO 320432; GO 6976; Isis 3521; or LY333531/LY379196.

Further anti-angiogenic compounds are e.g. thalidomide (THALOMID) and TNP-470.

Compounds which target, decrease or inhibit the activity of a protein or lipid phosphatase are, e.g. inhibitors of phosphatase 1, phosphatase 2A, PTEN or CDC25, e.g. okadaic acid or a derivative thereof.

Compounds which induce cell differentiation processes are, e.g. retinoic acid, α-, γ- or δ-tocopherol or α-, γ- or δ-tocotrienol.

The term cyclooxygenase inhibitor as used herein includes, but is not limited to, e.g. celecoxib (Celebrex^R), rofecoxib (Vioxx^R), etoricoxib, valdecoxib or a 5-alkyl-2-arylaminophenylacetic acid, e.g. 5-methyl-2-(2'-chloro-6'-fluoroanilino)phenyl acetic acid.

The term "histone deacetylase inhibitor" as used herein includes, but is not limited to MS-27-275, SAHA, pyroxamide, FR-901228 or valproic acid.

The term "bisphosphonates" as used herein includes, but is not limited to, etridonic, clodronic, tiludronic, pamidronic, alendronic, ibandronic, risedronic and zoledronic acid.

The term "matrix metalloproteinase inhibitor" as used herein includes, but is not limited to collagen peptidomimetic and non-petidomimetic inhibitors, tetracycline derivatives, e.g. hydroxamate peptidomimetic inhibitor batimastat and its orally bioavailable analogue marimastat, prinomastat, BMS-279251, BAY 12-9566, TAA211 or AAJ996.

The term "mTOR inhibitor" as used herein includes, but is not limited to rapamycin (sirolimus) or a derivative thereof, e.g. 32-deoxorapamycin, 16-pent-2-ynyloxy-32-deoxo-rapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-rapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin and, more preferably, 40-0-(2-hydroxyethyl)-rapamycin. Further examples of rapamycin derivatives include e.g. CCI779 or 40- [3-hydroxy-2-(hydroxy-

methyl)-2-methylpropanoate]-rapamycin or a pharmaceutically acceptable salt thereof, as disclosed in USP 5,362,718, ABT578 or 40-(tetrazolyl)-rapamycin, particularly 40-epi-(tetrazolyl)-rapamycin, e.g. as disclosed in WO 99/15530, or rapalogs as disclosed e.g. in WO 98/02441 and WO01/14387, e.g. AP23573.

Where the compounds of formula I are administered in conjunction with other immunosuppressive / immunomodulatory, anti-inflammatory or chemotherapeutic therapy, dosages of the co-administered immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic compound will of course vary depending on the type of co-drug employed, e.g. whether it is a steroid or a calcineurin inhibitor, on the specific drug employed, on the condition being treated and so forth.

In accordance with the foregoing the present invention provides in a yet further aspect:

5. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective non-toxic amount of a compound of formula I and at least a second drug substance, e.g. an immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic drug, e.g. as indicated above.

6. A pharmaceutical combination, e.g. a kit, comprising a) a first agent which is a compound of formula I as disclosed herein, in free form or in pharmaceutically acceptable salt form, and b) at least one co-agent, e.g. an immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic drug, e.g. as disclosed above. The kit may comprise instructions for its administration.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

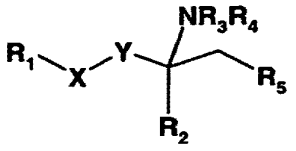
The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a compound of formula I and a co-agent, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a compound of formula I and a co-agent, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides

therapeutically effective levels of the 2 compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of 3 or more active ingredients.

In each case where citations of patent applications or scientific publications are given, the subject-matter relating to the compounds is hereby incorporated into the present application by reference. Comprised are likewise the pharmaceutically acceptable salts thereof, the corresponding racemates, diastereoisomers, enantiomers, tautomers as well as the corresponding crystal modifications of above disclosed compounds where present, e.g. solvates, hydrates and polymorphs, which are disclosed therein. The compounds used as active ingredients in the combinations of the invention can be prepared and administered as described in the cited documents, respectively. Also within the scope of this invention is the combination of more than two separate active ingredients as set forth above, i.e. a pharmaceutical combination within the scope of this invention could include three active ingredients or more. Further both the first agent and the co-agent are not the identical ingredient.

CLAIMS

1. A compound of formula I



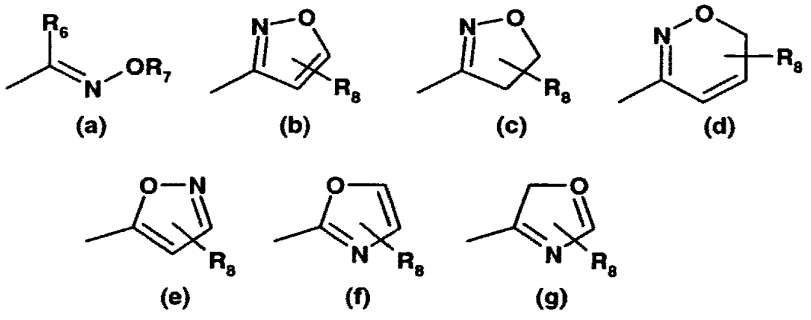
I

wherein

Y is $-CH_2CH_2-$, $-CH_2CH(OH)-$, $-CH(OH)CH_2-$, $-C(O)CH_2-$, $-CH_2C(O)-$, $-CH=CH-$; or 1,2-cyclopropylene;

X is arylene or C_{5-6}heteroarylene optionally substituted by one to three substituents selected from halogen, nitro, C_{1-10}alkyl and halogen-substituted C_{1-6}alkyl;

R_1 is aryl, aryl-C_{2-4}alkenyl, heteroaryl, or heteroaryl-C_{2-4}alkenyl each being substituted by (i) one to three substituents selected from hydrogen, halogen, amino, phenyl, heteroaryl, heteroaryl-C_{1-4}alkyl, C_{1-10}alkyl, cycloalkyl-C_{1-4}alkyl, cycloalkyl-C_{1-4}alkoxy, C_{1-10}alkoxy, C_{2-10}alkenyl, C_{2-10}alkynyl, C_{1-10}alkylthio, C_{1-10}alkylsulfonyl, C_{1-10}alkylsulfinyl, C_{1-4}alkyl-$S(O)_2NH$-, phenylC_{1-6}alkyl, or phenylC_{1-6}alkoxy, in each of which any aliphatic part of the group may be straight or branched chain and optionally substituted by up to three substituents selected from halogen, amino, hydroxy, cyano, or cycloalkyl groups and optionally interrupted by a double or triple bond or one or more C(O), NR_{12}, S, S(O), $S(O)_2$ or O groups, wherein R_{12} is hydrogen or C_{1-6}alkyl; and any aromatic group may be optionally substituted by one to three substituents selected from halogen, cyano, amino, C_{1-4}alkyl halogen-substituted-C_{1-4}alkyl and C_{1-8}alkoxy; and/or (ii) a group of formula (a), (b), (c), (d), (e), (f) or (g):

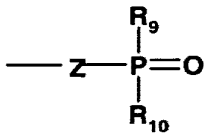


in which each of

R_6, R_7 and R_8 independently, is hydrogen; phenyl, C_{1-10}alkyl, cycloalkyl, heteroaryl, heteroaryl-C_{1-4}alkyl, C_{1-10}alkoxy, C_{2-10}alkenyl, C_{2-10}alkynyl, C_{1-10}alkylthio, C_{1-10}alkylsulfonyl, C_{1-10}alkylsulfinyl, phenylC_{1-8}alkyl, or phenylC_{1-6}alkoxy, in each of which any

aliphatic part of the group may be straight chain or branched and may be optionally substituted by up to three halogen, hydroxy, cycloalkyl, or C_{1-4}alkoxy groups and optionally interrupted by a double or triple bond or one or more C(O), NR_{12}, S, S(O), $S(O)_2$ or O groups, and any aromatic group may be optionally substituted by one to three substituents selected from halogen, CF_3, C_{1-8}alkyl and C_{1-8}alkoxy;

R_2 is hydrogen; halogen; C_{1-4}alkyl optionally substituted with one or more halogens; C_{2-6} alkenyl; C_{2-6}alkynyl; or cycloalkyl optionally substituted by halogen; aryl optionally substituted with hydroxy; or C_{1-4}alkyl optionally substituted on the terminal C atom by OH or a residue of formula (h):



(h)

in which Z is a direct bond, O, S, $(CH_2)_{1-2}$, CF_2, or NR_{11} where R_{11} is H, (C_{1-4})alkyl or halogen-substituted (C_{1-4})alkyl; and each of R_9 and R_{10}, independently, is H, OH, (C_{1-4})alkyl optionally substituted by one to three halogens, or (C_{1-4})alkoxy optionally substituted by halogen; with the proviso that R_9 and R_{10} are not both hydrogen;

each of R_3 and R_4, independently, is H or C_{1-4}alkyl optionally substituted by halogen or acyl; and R_5 is H, –OH, -Oacyl, –NHacyl, or a residue of formula (h) as defined above;

provided that at least either R_2 comprises a terminal OH or a residue of formula (h) or R_5 is OH or a residue of formula (h),

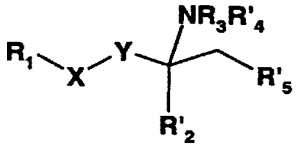
or a salt thereof.

2. A compound according to claim 1, wherein X is 1,4-phenylene, Y is $–CH_2-CH_2-$, R_1 is phenyl monosubstituted in position para by a group R_{15} wherein R_{15} is straight chain C_{5-8}alkyl; C_{2-8}alkenyl; or straight chain or branched C_{1-8}alkoxy optionally substituted by one C_{3-6}cycloalkyl or by a phenyl group optionally substituted by up to three halogens; or R_1 is para-monosubstituted phenyl substituted by a group of formula (a), (b) or (c), as defined in claim 1, R_2 is C_{1-4}alkyl optionally substituted on the terminal C atom by OH or a residue of formula (h) as defined above, R_3 and R_4 are hydrogen, and R_5 is OH, or a salt thereof.

3. A compound according to claim 1 or 2, wherein R_1 is phenyl monosubstituted in position para by a group of formula (a) as defined above, or a salt thereof.

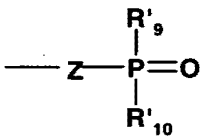
4. A compound according to any one of claims 1 to 3, wherein Z is O, or a salt thereof.

5. A process for preparing a compound according to any one of claims 1 to 5 which process comprises removing the hydrolysable groups present in a compound of formula II



II

wherein X, Y, R_1 and R_3 are as defined in claim 1, R_4' is an amino protecting group, R_2' has one of the significances given for R_2 above except that the terminal OH when present in the OH-substituted C_{1-4}alkyl is in protected form or the residue of formula (h) is replaced by a residue of formula (h') and R_5' is R_5" in which R_5" is H, -OH in protected form or a residue of formula (h'), provided that at least one of R_2' and R_5' is OH in protected form or a residue of formula (h'), the residue of formula (h') being:



(h')

wherein Z is as described above, and each of R_9' and R_{10}' is a hydrolysable group and, where required, converting the compounds of formula I obtained in free form into the desired salt form, or vice versa.

6. A compound according to any one of claims 1 to 4 or a pharmaceutically acceptable salt thereof for use as a pharmaceutical.

7. A compound according to any one of claims 1 to 4 or a pharmaceutically acceptable salt thereof for use in the preparation of a medicament.

8. A pharmaceutical composition comprising a compound according to any one of claims 1 to 4, or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable diluent or carrier therefor.

9. A pharmaceutical combination comprising a) a first agent which is a compound according to any one of claims 1 to 4, in free form or in pharmaceutically acceptable salt form, and b) at least one co-agent.

10. A method for preventing or treating disorders or diseases mediated by lymphocytes, for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases, for inhibiting or controlling deregulated

angiogenesis, or for preventing or treating diseases mediated by a neo-angiogenesis process or associated with deregulated angiogenesis in a subject comprising administering to the subject in need thereof an effective amount of a compound according to any one of claims 1 to 4, or a pharmaceutically acceptable salt thereof.

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(54) Title: USE OF EDG RECEPTOR BINDING AGENTS IN CANCER

(57) Abstract: Provided is a method for treating solid tumors, e.g. tumor invasiveness, and particularly inhibiting or controlling deregulated angiogenesis, using a sphingosine-1-phosphate receptor agonist, optionally in combination with a chemotherapeutic agent. The invention also comprises a combination of a sphingosine-1-phosphate receptor agonist with a chemotherapeutic agent.

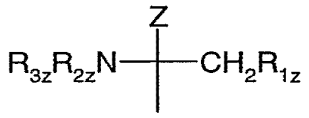
WO 03/097028 A1

Use of EDG receptor binding agents in cancer

The present invention relates to a new use for a sphingosine-1-phosphate (S1P) receptor agonist, particularly in the treatment of cancer.

S1P receptor agonists are accelerating lymphocyte homing (LH) agents which elicit a lymphopenia resulting from a re-distribution, preferably reversible, of lymphocytes from circulation to secondary lymphatic tissue, without evoking a generalized immunosuppression. Naïve cells are sequestered; CD4 and CD8 T-cells and B-cells from the blood are stimulated to migrate into lymph nodes (LN) and Peyer's patches (PP), and thus for example infiltration of cells into transplanted organs is inhibited.

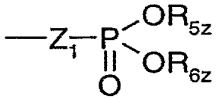
S1P receptor agonists are typically sphingosine analogues, such as 2-substituted 2-amino-propane-1,3-diol or 2-amino-propanol derivatives, e.g. a compound comprising a group of formula X



X

wherein

Z is H; C_{1-6}alkyl; C_{2-6}alkenyl; C_{2-6}alkynyl; phenyl; phenyl substituted by OH; C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH; or CH_2-R_{4z} wherein R_{4z} is OH, acyloxy or a residue of formula (a)



(a)

wherein Z_1 is a direct bond or O, preferably O; each of R_{5z} and R_{6z}, independently, is H, or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms;

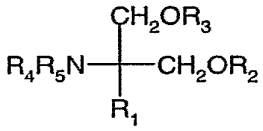
R_{1z} is OH, acyloxy or a residue of formula (a); and each of R_{2z} and R_{3z}, independently, is H, C_{1-4}alkyl or acyl.

Group of formula X is a functional group attached as a terminal group to a moiety which may be hydrophilic or lipophilic and comprise one or more aliphatic, alicyclic, aromatic and/or heterocyclic residues, to the extent that the resulting molecule wherein at least one of Z and R_{1z} is or comprises a residue of formula (a), signals as an agonist at one of more sphingosine-1-phosphate receptor.

S1P receptor agonists are compounds which signal as agonists at one or more sphingosine-1 phosphate receptors, e.g. S1P1 to S1P8. Agonist binding to a S1P receptor may e.g. result in dissociation of intracellular heterotrimeric G-proteins into Gα-GTP and Gβγ-GTP, and/or increased phosphorylation of the agonist-occupied receptor and activation of downstream signaling pathways/kinases. The binding affinity of S1P receptor agonists may be measured as described at paragraph **I.** below.

Examples of appropriate S1P receptor agonists are, for example:

- Compounds as disclosed in EP627406A1, e.g.a compound of formula I



I

wherein R_1 is a straight- or branched (C_{12-22})carbon chain

- which may have in the chain a bond or a hetero atom selected from a double bond, a triple bond, O, S, NR_6, wherein R_6 is H, alkyl, aralkyl, acyl or alkoxycarbonyl, and carbonyl, and/or
- which may have as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxyimino, hydroxy or carboxy; or

R_1 is

- a phenylalkyl wherein alkyl is a straight- or branched (C_{6-20})carbon chain; or
- a phenylalkyl wherein alkyl is a straight- or branched (C_{1-30})carbon chain wherein said phenylalkyl is substituted by
- a straight- or branched (C_{6-20})carbon chain optionally substituted by halogen,
- a straight- or branched (C_{6-20})alkoxy chain optionally substitued by halogen,
- a straight- or branched (C_{6-20})alkenyloxy,
- phenylalkoxy, halophenylalkoxy, phenylalkoxyalkyl, phenoxyalkoxy or phenoxyalkyl,
- cycloalkylalkyl substituted by C_{6-20}alkyl,
- heteroarylalkyl substituted by C_{6-20}alkyl,
- heterocyclic C_{6-20}alkyl or
- heterocyclic alkyl substituted by C_{2-20}alkyl,

and wherein

the alkyl moiety may have

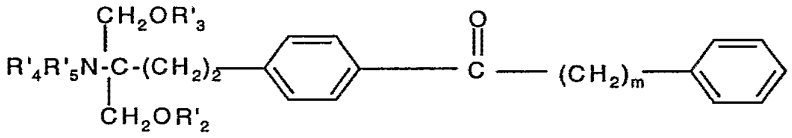
- in the carbon chain, a bond or a heteroatom selected from a double bond, a triple bond, O, S, sulfinyl, sulfonyl, or NR_6, wherein R_6 is as defined above, and

- as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxy or carboxy, and

each of R_2, R_3, R_4 and R_5, independently, is H, C_{1-4}alkyl or acyl

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in EP 1002792A1, e.g. a compound of formula II

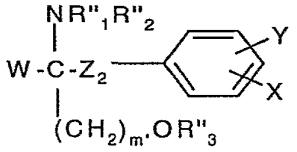


II

wherein m is 1 to 9 and each of R'$_2$, R'$_3$, R'$_4$ and R'$_5$, independently, is H, alkyl or acyl,

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in EP0778263 A1, e.g. a compound of formula III



III

wherein W is H; C_{1-6}alkyl, C_{2-6}alkenyl or C_{2-6}alkynyl; unsubstituted or by OH substituted phenyl; R"$_4$O(CH$_2$)$_n$; or C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH;

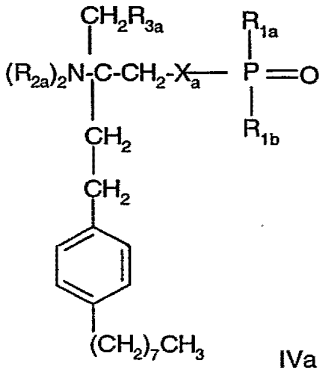
X is H or unsubstituted or substituted straight chain alkyl having a number p of carbon atoms or unsubstituted or substituted straight chain alkoxy having a number (p-1) of carbon atoms, e.g. substituted by 1 to 3 substitutents selected from the group consisting of C_{1-6} alkyl, OH, C_{1-6}alkoxy, acyloxy, amino, C_{1-6}alkylamino, acylamino, oxo, haloC_{1-6}alkyl, halogen, unsubstituted phenyl and phenyl substituted by 1 to 3 substituents selected from the group consisting of C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl and halogen; Y is H, C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl or halogen, Z_2 is a single bond or a straight chain alkylene having a number or carbon atoms of q,

each of p and q, independently, is an integer of 1 to 20, with the proviso of $6 \leq p+q \leq 23$, m' is 1, 2 or 3, n is 2 or 3,

each of R"$_1$, R"$_2$, R"$_3$ and R"$_4$, independently, is H, C_{1-4}alkyl or acyl,

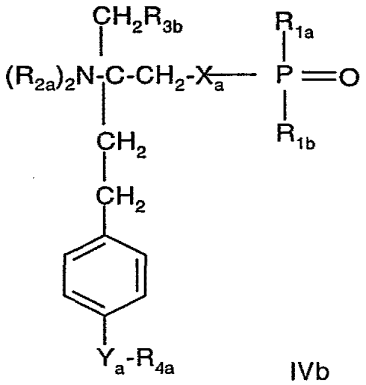
or a pharmaceutically acceptable salt thereof,

- Compounds as disclosed in WO02/18395, e.g. a compound of formula IVa or IVb



IVa

or

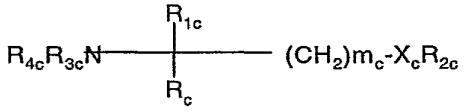


IVb

wherein X_a is O, S, NR_{1s} or a group $-(CH_2)_{na}-$, which group is unsubstituted or substituted by 1 to 4 halogen; n_a is 1 or 2, R_{1s} is H or (C_{1-4})alkyl, which alkyl is unsubstituted or substituted by halogen; R_{1a} is H, OH, (C_{1-4})alkyl or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by 1 to 3 halogen; R_{1b} is H, OH or (C_{1-4})alkyl, wherein alkyl is unsubstituted or substituted by halogen; each R_{2a} is independently selected from H or (C_{1-4})alkyl, which alkyl is unsubstituted or substitued by halogen; R_{3a} is H, OH, halogen or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; and R_{3b} is H, OH, halogen, (C_{1-4})alkyl wherein alkyl is unsubstituted or substituted by hydroxy, or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; Y_a is $-CH_2-$, -C(O)-, -CH(OH)-, -C(=NOH)-, O or S, and R_{4a} is (C_{4-14})alkyl or (C_{4-14})alkenyl;

or a pharmaceutically acceptable salt or hydrate thereof;

- Compounds as disclosed in WO 02/076995, e.g. a compound of formula V



V

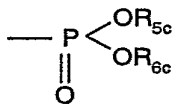
wherein

m_c is 1, 2 or 3;

X_c is O or a direct bond;

R_{1c} is H; C_{1-6} alkyl optionally substituted by OH, acyl, halogen, C_{3-10}cycloalkyl, phenyl or hydroxy-phenylene; C_{2-6}alkenyl; C_{2-6}alkynyl; or phenyl optionally substituted by OH;

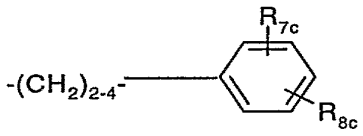
R_{2c} is



wherein R_{5c} is H or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms, and R_{6c} is H or C_{1-4}alkyl optionally substituted by halogen;

each of R_{3c} and R_{4c}, independently, is H, C_{1-4}alkyl optionally substituted by halogen, or acyl, and

R_c is C_{13-20}alkyl which may optionally have in the chain an oxygen atom and which may optionally be substituted by nitro, halogen, amino, hydroxy or carboxy; or a residue of formula (a)

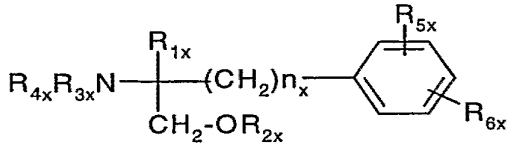


(a)

wherein R_{7c} is H, C_{1-4}alkyl or C_{1-4}alkoxy, and R_{8c} is substituted C_{1-20}alkanoyl, phenylC_{1-14}alkyl wherein the C_{1-14}alkyl is optionally substituted by halogen or OH, cycloalkylC_{1-14}alkoxy or phenylC_{1-14}alkoxy wherein the cycloalkyl or phenyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy, phenylC_{1-14}alkoxy-C_{1-14}alkyl, phenoxyC_{1-14}alkoxy or phenoxyC_{1-14}alkyl,

R_c being also a residue of formula (a) wherein R_{8c} is C_{1-14}alkoxy when R_{1c} is C_{1-4}alkyl, C_{2-6}alkenyl or C_{2-6}alkynyl,

or a compound of formula VI



VI

wherein

n_x is 2, 3 or 4

R_{1x} is H; C_{1-6}alkyl optionally substituted by OH, acyl, halogen, cycloalkyl, phenyl or hydroxy-phenylene; C_{2-6}alkenyl; C_{2-6}alkynyl; or phenyl optionally substituted by OH;

R_{2x} is H, C_{1-4} alkyl or acyl

each of R_{3x} and R_{4x}, independently is H, C_{1-4}alkyl optionally substituted by halogen or acyl,

R_{5x} is H, C_{1-4}alkyl or C_{1-4}alkoxy, and

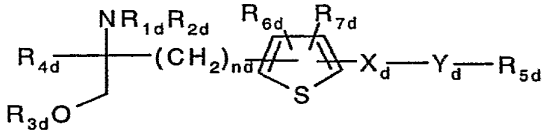
R_{6x} is C_{1-20}alkanoyl substituted by cycloalkyl; cyloalkylC_{1-14}alkoxy wherein the cycloalkyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy; phenylC_{1-14}alkoxy wherein the phenyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy,

R_{6x} being also C_{4-14}alkoxy when R_{1x} is C_{2-4}alkyl substituted by OH, or pentyloxy or hexyloxy when R_{1x} is C_{1-4}akyl,

provided that R_{6x} is other than phenyl-butylenoxy when either R_{5x} is H or R_{1x} is methyl,

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in WO02/06268Al, e.g. a compound of formula VII



VII

wherein each of R_{1d} and R_{2d}, independently, is H or an amino-protecting group;

R_{3d} is hydrogen or a hydroxy-protecting group;

R_{4d} is lower alkyl;

n_d is an integer of 1 to 6;

X_d is ethylene, vinylene, ethynylene, a group having a formula – D-CH_2- (wherein D is carbonyl, – CH(OH)-, O, S or N), aryl or aryl substituted by up to three substitutents selected from group a as defined hereinafter;

Y_d is single bond, C_{1-10}alkylene, C_{1-10}alkylene which is substituted by up to three substitutents selected from groups a and b, C_{1-10}alkylene having O or S in the middle or end of the carbon chain, or C_{1-10}alkylene having O or S in the middle or end of the carbon chain which is substituted by up to three substituents selected from groups a and b;

R_{5d} is hydrogen, cycloalkyl, aryl, heterocycle, cycloalkyl substituted by up to three substituents selected from groups a and b, aryl substituted by up to three substituents selected from groups a and b, or heterocycle substituted by up to three substituents selected from groups a and b; and

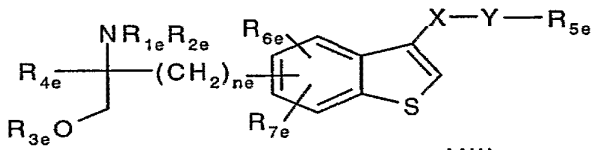
each of R_{6d} and R_{7d}, independently, is H or a substituent selected from group a;

<group a > is halogen, lower alkyl, halogeno lower alkyl, lower alkoxy, lower alkylthio, carboxyl, lower alkoxycarbonyl, hydroxy, lower aliphatic acyl, amino, mono-lower alkylamino, di-lower alkylamino, lower aliphatic acylamino, cyano or nitro;

<group b > is cycloalkyl, aryl, heterocycle, each being optionally substituted by up to three substituents selected from group a;

with the proviso that when R_{5d} is hydrogen, Y_d is a either a single bond or linear C_{1-10} alkylene, or a pharmacologically acceptable salt or ester thereof.

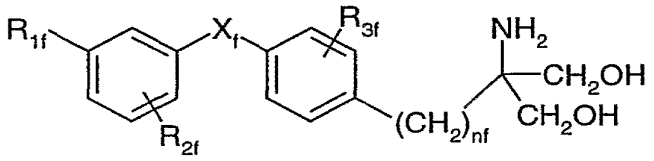
-Compounds as disclosed in JP-14316985 (JP2002316985), e.g. a compound of formula VIII:



VIII

wherein R_{1e},R_{2e},R_{3e},R_{4e},R_{5e},R_{6e},R_{7e}, n_e, X_e and Y_e are as disclosed in JP-14316985; or a pharmacologically acceptable salt or ester thereof.

-Compounds as disclosed in WO 03/29184 and WO 03/29205, e.g. compounds of formula IX



IX

wherein X_f is O or S, and R_{1f}, R_{2f}, R_{3f} and n_f are as disclosed in WO 03/29184 and O3/29205, e.g. 2-amino-2-[4-(3-benzyloxyphenoxy)-2-chlorophenyl]propyl-1,3-propane-diol or 2-amino-2-[4-(benzyloxyphenylthio)-2- chlorophenyl]propyl-1,3-propane-diol.

In each case where citations of patent applications are given, the subject matter relating to the compounds is hereby incorporated into the present application by reference.

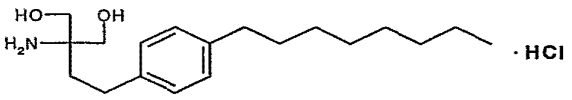
Acyl may be a residue R_y-CO- wherein R_y is C_{1-6}alkyl, C_{3-6}cycloalkyl, phenyl or phenyl-C_{1-4}alkyl. Unless otherwise stated, alkyl, alkoxy, alkenyl or alkynyl may be straight or branched.

When in the compounds of formula I the carbon chain as R_1 is substituted, it is preferably substituted by halogen, nitro, amino, hydroxy or carboxy. When the carbon chain is interrupted by an optionally substituted phenylene, the carbon chain is preferably unsubstituted. When the phenylene moiety is substituted, it is preferably substituted by halogen, nitro, amino, methoxy, hydroxy or carboxy.

Preferred compounds of formula I are those wherein R_1 is C_{13-20}alkyl, optionally substituted by nitro, halogen, amino, hydroxy or carboxy, and, more preferably those wherein R_1 is phenylalkyl substituted by C_{6-14}-alkyl chain optionally substituted by halogen and the alkyl moiety is a C_{1-6}alkyl optionally substituted by hydroxy. More preferably, R_1 is phenyl-C_{1-6}alkyl substituted on the phenyl by a straight or branched, preferably straight, C_{6-14}alkyl chain. The C_{6-14}alkyl chain may be in ortho, meta or para, preferably in para.

Preferably each of R_2 to R_5 is H.

A preferred compound of formula I is 2-amino-2-tetradecyl-1,3-propanediol. A particularly preferred S1P receptor agonist of formula I is FTY720, i.e. 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol in free form or in a pharmaceutically acceptable salt form (referred to hereinafter as Compound A), e.g. the hydrochloride, as shown:



A preferred compound of formula II is the one wherein each of R'_2 to R'_5 is H and m is 4, i.e. 2-amino-2-{2-[4-(1-oxo-5-phenylpentyl)phenyl]ethyl}propane-1,3-diol, in free form or in pharmaceutically acceptable salt form (referred to hereinafter as Compound B), e.g the hydrochloride.

A preferred compound of formula III is the one wherein W is CH_3, each of R''_1 to R''_3 is H, Z_2 is ethylene, X is heptyloxy and Y is H, i.e. 2-amino-4-(4-heptyloxyphenyl)-2-methyl-butanol, in free form or in pharmaceutically acceptable salt form (referred to hereinafter as Compound C), e.g. the hydrochloride. The R-enantiomer is particularly preferred.

A preferred compound of formula IVa is the FTY720-phosphate (R_{2a} is H, R_{3a} is OH, X_a is O, R_{1a} and R_{1b} are OH). A preferred compound of formula IVb is the Compound C-phosphate (R_{2a} is H, R_{3b} is OH, X_a is O, R_{1a} and R_{1b} are OH, Y_a is O and R_{4a} is heptyl). A preferred compound of formula V is Compound B-phosphate.

A preferred compound of formula V is phosphoric acid mono-[(R)-2-amino-2-methyl-4-(4-pentyloxy-phenyl)-butyl]ester.

A preferred compound of formula VIII is (2R)-2-amino-4-[3-(4-cyclohexyloxybutyl)benzo[b]thien-6-yl]-2-methylbutan-1-ol.

When the compounds of formulae I to IX have one or more asymmetric centers in the molecule, the present invention is to be understood as embracing the various optical isomers, as well as racemates, diastereoisomers and mixtures thereof are embraced. Compounds of formula III or IVb, when the carbon atom bearing the amino group is asymmetric, have preferably the R-configuration at this carbon atom.

Examples of pharmaceutically acceptable salts of the compounds of the formulae I to IX include salts with inorganic acids, such as hydrochloride, hydrobromide and sulfate, salts with organic acids, such as acetate, fumarate, maleate, benzoate, citrate, malate,

methanesulfonate and benzenesulfonate salts, or, when appropriate, salts with metals such as sodium, potassium, calcium and aluminium, salts with amines, such as triethylamine and salts with dibasic amino acids, such as lysine. The compounds and salts of the methods of the present invention encompass hydrate and solvate forms.

The S1P receptor agonists have, on the basis of observed activity, e.g. homing of lymphocytes, e.g. as described in EP627406A1 or USP 6,004,565, been found to be useful e.g. as immunosuppressant, e.g. in the treatment of acute allograft rejection. It has now been found that S1P receptor agonists have interesting properties which make them useful for cancer chemotherapy, particularly of solid tumors, especially of advanced solid tumors. There is still the need to expand the armamentarium of cancer treatment of solid tumors, especially in cases where treatment with anticancer compounds is not associated with disease regression or stabilization.

In accordance with the particular findings of the present invention, there is provided:

1.1 A method for treating solid tumors in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.2 A method for inhibiting growth of solid tumors in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.3 A method for inducing tumor regression, e.g. tumor mass reduction, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.4 A method for treating solid tumor invasiveness or symptoms associated with such tumor growth in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.5 A method for preventing metastatic spread of tumours or for preventing or inhibiting growth of micrometastasis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.6 A method for inhibiting or controlling deregulated angiogenesis, e.g. sphingosine-1-phosphate (S1P) mediated angiogenesis, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.7 A method for preventing or treating diseases mediated by a neo-angiogenesis process or associated with deregulated angiogenesis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

By "solid tumors" are meant tumors and/or metastasis (whereever located) other than lymphatic cancer, e.g. brain and other central nervous system tumors (eg. tumors of the meninges, brain, spinal cord, cranial nerves and other parts of central nervous system, e.g. glioblastomas or medulla blastomas); head and/or neck cancer; breast tumors; circulatory system tumors (e.g. heart, mediastinum and pleura, and other intrathoracic organs, vascular tumors and tumor-associated vascular tissue); excretory system tumors (e.g. kidney, renal pelvis, ureter, bladder, other and unspecified urinary organs); gastrointestinal tract tumors (e.g. oesophagus, stomach, small intestine, colon, colorectal, rectosigmoid junction, rectum, anus and anal canal), tumors involving the liver and intrahepatic bile ducts, gall bladder, other and unspecified parts of biliary tract, pancreas, other and digestive organs); oral cavity (lip, tongue, gum, floor of mouth, palate, and other parts of mouth, parotid gland, and other parts of the salivary glands, tonsil, oropharynx, nasopharynx, pyriform sinus, hypopharynx, and other sites in the lip, oral cavity and pharynx); reproductive system tumors (e.g. vulva, vagina, Cervix uteri, Corpus uteri, uterus, ovary, and other sites associated with female genital organs, placenta, penis, prostate, testis, and other sites associated with male genital organs); respiratory tract tumors (e.g. nasal cavity and middle ear, accessory sinuses, larynx, trachea, bronchus and lung, e.g. small cell lung cancer or non-small cell lung cancer); skeletal system tumors (e.g. bone and articular cartilage of limbs, bone articular cartilage and other sites); skin tumors (e.g. malignant melanoma of the skin, non-melanoma skin cancer, basal cell carcinoma of skin, squamous cell carcinoma of skin, mesothelioma, Kaposi's sarcoma); and tumors involving other tissues incluing peripheral nerves and autonomic nervous system, connective and soft tissue, retroperitoneum and peritoneum, eye and adnexa, thyroid, adrenal gland and other endocrine glands and related structures, secondary and unspecified malignant neoplasm of lymph nodes, secondary malignant neoplasm of respiratory and digestive systems and secondary malignant neoplasm of other sites.

Where hereinbefore and subsequently a tumor, a tumor disease, a carcinoma or a cancer is mentioned, also metastasis in the original organ or tissue and/or in any other location are implied alternatively or in addition, whatever the location of the tumor and/or metastasis is.

When the S1P receptor agonist is a compound of formula I, e.g. Compound A, or a compound of formula IVa or IVb, in one embodiment it is used in the treatment methods 1.1, 1.2, 1.3 or 1.4 for a solid tumor other than breast, prostate, bladder, kidney or lung tumor.

In a series of further specific or alternative embodiments, the present invention also provides

1.8 A method for enhancing the activity of a chemotherapeutic agent or for overcoming resistance to a chemotherapeutic agent in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist , e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, either concomitantly or sequentially with said chemotherapeutic agent.

1.9 A method according to 1.8 wherein the chemotherapeutic agent is an inhibitor of signal transduction pathways directed either against host cells or processes involved in tumor formation and/or metastases formation or utilised by tumour cells for proliferation, survival, differentiation or development of drug resistance.

1.10 A method as indicated above, wherein the S1P receptor agonist is administered intermittently.

In a series of further specific or alternative embodiments, the present invention also provides:

2.1 A S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, for use in any method as defined under 1.1 to 1.4 above, preferably for a solid tumor other than breast, prostate, bladder, kidney or lung when the S1P receptor agonist is a compound of formula I, e.g. Compound A, or a compound of formula IVa or IVb.

2.2 A S1P receptor agonist, e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, for use in any method as defined under 1.5 to 1.10 above or 7 below.

3.1 A S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, for use in the preparation of a pharmaceutical composition for use in any method as defined under 1.1 to 1.4 above, preferably for a solid tumor other than breast, prostate, bladder, kidney or lung when the S1P receptor agonist is a compound of formula I, e.g. Compound A, or a compound of formula IVa or IVb.

3.2 A S1P receptor agonist, e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, for use in the preparation of a pharmaceutical composition for use in any method as defined under 1.5 to 1.10 above or 7 below.

4.1 A pharmaceutical composition for use in any method as defined under 1.1 to 1.4 above comprising a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically acceptable diluents or carriers therefor, preferably for a solid tumor other than breast, prostate, bladder, kidney or lung when the S1P receptor agonist is a compound of formula I, e.g. Compound A, or a compound of formula IVa or IVb.

4.2 A pharmaceutical composition for use in any method as defined under 1.5 to 1.10 above or 7 below comprising a S1P receptor agonist, e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically acceptable diluents or carriers therefor.

5.1 A pharmaceutical combination comprising a) a first agent which is a S1P receptor agonist , e.g. a S1P receptor agonist comprising a group of formula X or a pharmaceutically acceptable salt thereof and b) a co-agent which is a chemotherapeutic agent, e.g. as defined hereinafter.

5.2 A pharmaceutical combination comprising an amount of a) a first agent which is a S1P receptor agonist , e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, and b) a co-agent which is a chemotherapeutic agent selected from the compounds defined under section xi) below, to produce a synergistic therapeutic effect.

6. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of a S1P receptor agonist, e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, and a second drug substance, said second drug substance being a chemotherapeutic agent, e.g. as indicated hereinafter.

7. A method for treating lymphoproliferative or myeloproliferative disorders, e.g. for treating tumor invasiveness or symptoms associated with such tumor growth in a subject in need thereof, comprising co-administering to said subject, e.g. concomitantly or in sequence, of a S1P receptor agonist, e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, and a second drug substance, said second drug substance being a chemotherapeutic agent, e.g. as indicated hereinafter.

By "lymphatic cancer" are meant e.g. tumors of blood and lymphatic system (e.g. Hodgkin's disease, Non-Hodgkin's lymphoma, Burkitt's lymphoma, AIDS-related lymphomas, malignant immunoproliferative diseases, multiple myeloma and malignant plasma cell neoplasms, lymphoid leukemia, acute or chronic myeloid leukemia, acute or chronic lymphocytic leukemia, monocytic leukemia, other leukemias of specified cell type, leukemia of

unspecified cell type, other and unspecified malignant neoplasms of lymphoid, haematopoietic and related tissues, for example diffuse large cell lymphoma, T-cell lymphoma or cutaneous T-cell lymphoma). Myeloid cancer includes e.g. acute or chronic myeloid leukaemia.

By the term "chemotherapeutic agent" is meant especially any chemotherapeutic agent other than the S1P receptor agonist. It includes but is not limited to,

i. an aromatase inhibitor,
ii. an antiestrogen, an anti-androgen (especially in the case of prostate cancer) or a gonadorelin agonist,
iii. a topoisomerase I inhibitor or a topoisomerase II inhibitor,
iv. a microtubule active agent, an alkylating agent, an antineoplastic antimetabolite or a platin compound,
v. a compound targeting/decreasing a protein or lipid kinase activity or a protein or lipid phosphatase activity, a further anti-angiogenic compound or a compound which induces cell differentiation processes,
vi. a bradykinin 1 receptor or an angiotensin II antagonist,
vii. a cyclooxygenase inhibitor, a bisphosphonate, a histone deacetylase inhibitor, a heparanase inhibitor (prevents heparan sulphate degradation), e.g. PI-88, a biological response modifier, preferably a lymphokine or interferons, e.g. interferon γ, an ubiquitination inhibitor, or an inhibitor which blocks anti-apoptotic pathways,
viii. an inhibitor of Ras oncogenic isoforms, e.g. H-Ras, K-Ras or N-Ras, or a farnesyl transferase inhibitor, e.g. L-744,832 or DK8G557,
ix. a telomerase inhibitor, e.g. telomestatin,
x. a protease inhibitor, a matrix metalloproteinase inhibitor, a methionine aminopeptidase inhibitor, e.g. bengamide or a derivative thereof, or a proteosome inhibitor, e.g. PS-341, and/or
xi) a mTOR inhibitor.

The term "aromatase inhibitor" as used herein relates to a compound which inhibits the estrogen production, i.e. the conversion of the substrates androstenedione and testosterone to estrone and estradiol, respectively. The term includes, but is not limited to steroids, especially atamestane, exemestane and formestane and, in particular, non-steroids, especially aminoglutethimide, roglethimide, pyridoglutethimide, trilostane, testolactone, ketokonazole, vorozole, fadrozole, anastrozole and letrozole. Exemestane can be administered, e.g., in the form as it is marketed, e.g. under the trademark AROMASIN™. Formestane can be administered, e.g., in the form as it is marketed, e.g. under the

trademark LENTARON™. Fadrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark AFEMA™. Anastrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark ARIMIDEX™. Letrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark FEMARA™ or FEMAR™ Aminoglutethimide can be administered, e.g., in the form as it is marketed, e.g. under the trademark ORIMETEN™. A combination of the invention comprising a chemotherapeutic agent which is an aromatase inhibitor is particularly useful for the treatment of hormone receptor positive tumors, e.g. breast tumors.

The term "antiestrogen" as used herein relates to a compound which antagonizes the effect of estrogens at the estrogen receptor level. The term includes, but is not limited to tamoxifen, fulvestrant, raloxifene and raloxifene hydrochloride. Tamoxifen can be administered, e.g., in the form as it is marketed, e.g. under the trademark NOLVADEX™. Raloxifene hydrochloride can be administered, e.g., in the form as it is marketed, e.g. under the trademark EVISTA™. Fulvestrant can be formulated as disclosed in US 4,659,516 or it can be administered, e.g., in the form as it is marketed, e.g. under the trademark FASLODEX™. A combination of the invention comprising a chemotherapeutic agent which is an antiestrogen is particularly useful for the treatment of estrogen receptor positive tumors, e.g. breast tumors.

The term "anti-androgen" as used herein relates to any substance which is capable of inhibiting the biological effects of androgenic hormones and includes, but is not limited to, bicalutamide (CASODEX™), which can be formulated, e.g. as disclosed in US 4,636,505.

The term "gonadorelin agonist" as used herein includes, but is not limited to abarelix, goserelin and goserelin acetate. Goserelin is disclosed in US 4,100,274 and can be administered, e.g., in the form as it is marketed, e.g. under the trademark ZOLADEX™. Abarelix can be formulated, eg. as disclosed in US 5,843,901.

The term "topoisomerase I inhibitor" as used herein includes, but is not limited to topotecan, irinotecan, 9-nitrocamptothecin and the macromolecular camptothecin conjugate PNU-166148 (compound A1 in WO99/17804). Irinotecan can be administered, e.g. in the form as it is marketed, e.g. under the trademark CAMPTOSAR™. Topotecan can be administered, e.g., in the form as it is marketed, e.g. under the trademark HYCAMTIN™.

The term "topoisomerase II inhibitor" as used herein includes, but is not limited to the anthracyclines such as doxorubicin (including liposomal formulation, e.g. CAELYX™), daunorubicin, epirubicin, idarubicin and nemorubicin, the anthraquinones mitoxantrone and losoxantrone, and the podophillotoxines etoposide and teniposide. Etoposide can be

administered, e.g. in the form as it is marketed, e.g. under the trademark ETOPOPHOS™. Teniposide can be administered, e.g. in the form as it is marketed, e.g. under the trademark VM 26-BRISTOL™ Doxorubicin can be administered, e.g. in the form as it is marketed, e.g. under the trademark ADRIBLASTIN™. Epirubicin can be administered, e.g. in the form as it is marketed, e.g. under the trademark FARMORUBICIN™. Idarubicin can be administered, e.g. in the form as it is marketed, e.g. under the trademark ZAVEDOS™. Mitoxantrone can be administered, e.g. in the form as it is marketed, e.g. under the trademark NOVANTRON™.

The term "microtubule active agent" relates to microtubule stabilizing and microtubule destabilizing agents including, but not limited to taxanes, e.g. paclitaxel and docetaxel, vinca alkaloids, e.g., vinblastine, especially vinblastine sulfate, vincristine especially vincristine sulfate, and vinorelbine, discodermolides and epothilones and derivatives thereof, e.g. epothilone B or a derivative thereof. Paclitaxel may be administered e.g. in the form as it is marketed, e.g. TAXOL™. Docetaxel can be administered, e.g., in the form as it is marketed, e.g. under the trademark TAXOTERE™. Vinblastine sulfate can be administered, e.g., in the form as it is marketed, e.g. under the trademark VINBLASTIN R.P.™. Vincristine sulfate can be administered, e.g., in the form as it is marketed, e.g. under the trademark FARMISTIN™. Discodermolide can be obtained, e.g., as disclosed in US 5,010,099.

The term "alkylating agent" as used herein includes, but is not limited to busulfan, chlorambucil, cyclophosphamide, ifosfamide, melphalan or nitrosourea (BCNU or Gliadel™). Cyclophosphamide can be administered, e.g., in the form as it is marketed, e.g. under the trademark CYCLOSTIN™. Ifosfamide can be administered, e.g., in the form as it is marketed, e.g. under the trademark HOLOXAN™.

The term "antineoplastic antimetabolite" includes, but is not limited to 5-fluorouracil, capecitabine, gemcitabine, cytarabine, fludarabine, thioguanine, methotrexate and edatrexate. Capecitabine can be administered, e.g., in the form as it is marketed, e.g. under the trademark XELODA™. Gemcitabine can be administered, e.g., in the form as it is marketed, e.g. under the trademark GEMZAR™.

The term "platin compound" as used herein includes, but is not limited to carboplatin, cis-platin and oxaliplatin. Carboplatin can be administered, e.g., in the form as it is marketed, e.g. under the trademark CARBOPLAT™. Oxaliplatin can be administered, e.g., in the form as it is marketed, e.g. under the trademark ELOXATIN™.

The term "compounds targeting/decreasing a protein or lipid kinase activity or further anti-angiogenic compounds" as used herein includes, but is not limited to protein tyrosine kinase

and/or serine and/or threonine kinase inhibitors or lipid kinase inhibitors, e.g. compounds targeting, decreasing or inhibiting the activity of the epidermal growth factor family of receptor tyrosine kinases (EGFR, ErbB2, ErbB3, ErbB4 as homo- or heterodimers), the vascular endothelial growth factor family of receptor tyrosine kinases (VEGFR), the platelet-derived growth factor-receptors (PDGFR), the fibroblast growth factor-receptors (FGFR), the insulin-like growth factor receptor 1 (IGF-1R), the Trk receptor tyrosine kinase family, the Axl receptor tyrosine kinase family, the Ret receptor tyrosine kinase, the Kit/SCFR receptor tyrosine kinase, members of the c-Abl family and their gene-fusion products (e.g. BCR-Abl), members of the protein kinase C (PKC) and Raf family of serine/threonine kinases, members of the MEK, SRC, JAK, FAK, PDK or PI(3) kinase family, or of the PI(3)-kinase-related kinase family, and/or members of the cyclin-dependent kinase family (CDK) and anti-angiogenic compounds having another mechanism for their activity, e.g. unrelated to protein or lipid kinase inhibition.

Compounds which target, decrease or inhibit the activity of VEGFR are especially compounds, proteins or antibodies which inhibit the VEGF receptor tyrosine kinase, inhibit a VEGF receptor or bind to VEGF, and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 98/35958, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or a pharmaceutically acceptable salt thereof, e.g. the succinate, in WO 00/27820, e.g. a N-aryl(thio) anthranilic acid amide derivative e.g. 2-[(4-pyridyl)methyl]amino-N-[3-methoxy-5-(trifluoromethyl)phenyl]benzamide or 2-[(1-oxido-4-pyridyl)methyl]amino-N-[3-trifluoromethylphenyl]benzamide, or in WO 00/09495, WO 00/59509, WO 98/11223, WO 00/27819 and EP 0 769 947; those as described by M. Prewett et al in Cancer Research 59 (1999) 5209-5218, by F. Yuan et al in Proc. Natl. Acad. Sci. USA, vol. 93, pp. 14765-14770, Dec. 1996, by Z. Zhu et al in Cancer Res. 58, 1998, 3209-3214, and by J. Mordenti et al in Toxicologic Pathology, Vol. 27, no. 1, pp 14-21, 1999; in WO 00/37502 and WO 94/10202; Angiostatin™, described by M. S. O'Reilly et al, Cell 79, 1994, 315-328; Endostatin™, described by M. S. O'Reilly et al, Cell 88, 1997, 277-285; anthranilic acid amides; ZD4190; ZD6474; SU5416; SU6668; or anti-VEGF antibodies or anti-VEGF receptor antibodies,e.g. RhuMab.

By antibody is meant intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies formed from at least 2 intact antibodies, and antibodies fragments so long as they exhibit the desired biological activity.

Compounds which target, decrease or inhibit the activity of the epidermal growth factor receptor family are especially compounds, proteins or antibodies which inhibit members of the EGF receptor tyrosine kinase family, e.g. EGF receptor, ErbB2, ErbB3 and ErbB4 or bind

to EGF or EGF related ligands, or which have a dual inhibiting effect on the ErbB and VEGF receptor kinase and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 97/02266, e.g. the compound of ex. 39, or in EP 0 564 409, WO 99/03854, EP 0520722, EP 0 566 226, EP 0 787 722, EP 0 837 063, US 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and, especially, WO 96/30347 (e.g. compound known as CP 358774), WO 96/33980 (e.g. compound ZD 1839) and WO 95/03283 (e.g. compound ZM105180) or PCT/EP02/08780; e.g. trastuzumab (Herpetin^{R}), cetuximab, Iressa, OSI-774, CI-1033, EKB-569, GW-2016, E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6.3 or E7.6.3.

Compounds which target, decrease or inhibit the activity of PDGFR are especially compounds which inhibit the PDGF receptor, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib.

Compounds which target, decrease or inhibit the activity of c-Abl family members and their gene fusion products, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib; PD180970; AG957; or NSC 680410.

Compounds which target, decrease or inhibit the activity of protein kinase C, Raf, MEK, SRC, JAK, FAK and PDK family members, or PI(3) kinase or PI(3) kinase-related family members, and/or members of the cyclin-dependent kinase family (CDK) are especially those staurosporine derivatives disclosed in EP 0 296 110, e.g. midostaurin; examples of further compounds include e.g. UCN-01, safingol, BAY 43-9006, Bryostatin 1, Perifosine; UO126; Ilmofosine; RO 318220 and RO 320432; GO 6976; Isis 3521; or LY333531/LY379196.

Further anti-angiogenic compounds are e.g. thalidomide (THALOMID) and TNP-470.

Compounds which target, decrease or inhibit the activity of a protein or lipid phosphatase are e.g. inhibitors of phosphatase 1, phosphatase 2A, PTEN or CDC25, e.g. okadaic acid or a derivative thereof.

Compounds which induce cell differentiation processes are e.g. retinoic acid, α–, γ– or δ-tocopherol or α–, γ– or δ-tocotrienol.

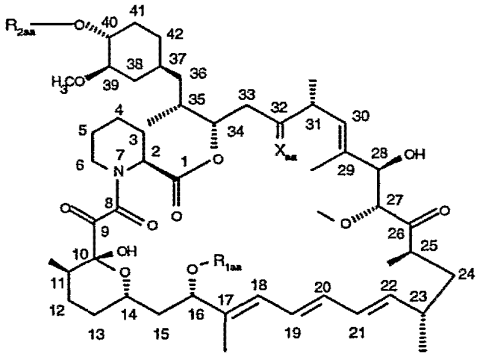
The term cyclooxygenase inhibitor as used herein includes, but is not limited to, e.g. celecoxib (Celebrex^{R}), rofecoxib (Vioxx^{R}), etoricoxib, valdecoxib or a 5-alkyl-2-arylaminophenylacetic acid, e.g. 5-methyl-2-(2'-chloro-6'-fluoroanilino)phenyl acetic acid.

The term "histone deacetylase inhibitor" as used herein includes, but is not limited to MS-27-275, SAHA, pyroxamide, FR-901228 or valproic acid.

The term "bisphosphonates" as used herein includes, but is not limited to, etridonic, clodronic, tiludronic, pamidronic, alendronic, ibandronic, risedronic and zoledronic acid. "Etridonic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark DIDRONEL™. "Clodronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark BONEFOS™. "Tiludronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark SKELID™. "Pamidronic acid" can be administered, e.g. in the form as it is marketed, e.g. under the trademark AREDIA™. "Alendronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark FOSAMAX™. "Ibandronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark BONDRANAT™. "Risedronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark ACTONEL™. "Zoledronic acid" can be administered, e.g. in the form as it is marketed, e.g. under the trademark ZOMETA™

The term "matrix metalloproteinase inhibitor" as used herein includes, but is not limited to collagen peptidomimetic and nonpetidomimetic inhibitors, tetracycline derivatives, e.g. hydroxamate peptidomimetic inhibitor batimastat and its orally bioavailable analogue marimastat, prinomastat, BMS-279251, BAY 12-9566, TAA211 or AAJ996.

The term "mTOR inhibitor" as used herein includes, but is not limited to rapamycin (sirolimus) or a derivative thereof. Rapamycin is a known macrolide antibiotic produced by Streptomyces hygroscopicus. Suitable derivatives of rapamycin include e.g. compounds of formula A



A

wherein

R_{1aa} is CH_3 or C_{3-6}alkynyl,

R_{2aa} is H or $-CH_2-CH_2-OH$, 3-hydroxy-2-(hydroxymethyl)-2-methyl-propanoyl or tetrazolyl, and

X_{aa} is =O, (H,H) or (H,OH)

provided that R_{2aa} is other than H when X_{aa} is =O and R_{1aa} is CH_3.

or a prodrug thereof when R_{2aa} is $-CH_2-CH_2-OH$, e.g. a physiologically hydrolysable ether thereof.

Compounds of formula A are disclosed e.g. in WO 94/09010, WO 95/16691, WO 96/41807, USP 5,362,718 or WO 99/15530 which are incorporated herein by reference. They may be prepared as diclosed or by analogy to the procedures described in these references

Preferred rapamycin derivatives are 32-deoxorapamycin, 16-pent-2-ynyloxy-32-deoxorapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-rapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin and, more preferably, 40-0-(2-hydroxyethyl)-rapamycin. Further examples of rapamycin derivatives include e.g. CCI779 or 40- [3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate]-rapamycin or a pharmaceutically acceptable salt thereof, as disclosed in USP 5,362,718, ABT578 or 40-(tetrazolyl)-rapamycin, particularly 40-epi-(tetrazolyl)-rapamycin, e.g. as disclosed in WO 99/15530, or rapalogs as disclosed e.g. in WO 98/02441 and WO01/14387, e.g. AP23573.

In each case where citations of patent applications or scientific publications are given, the subject-matter relating to the compounds is hereby incorporated into the present application by reference. Comprised are likewise the pharmaceutically acceptable salts thereof, the corresponding racemates, diastereoisomers, enantiomers, tautomers as well as the corresponding crystal modifications of above disclosed compounds where present, e.g. solvates, hydrates and polymorphs, which are disclosed therein. The compounds used as active ingredients in the combinations of the invention can be prepared and administered as described in the cited documents, respectively. Also within the scope of this invention is the combination of more than two separate active ingredients as set forth above, i.e. a pharmaceutical combination within the scope of this invention could include three active ingredients or more. Further both the first agent and the co-agent are not the identical ingredient.

Utility of the S1P agonists, e.g. the S1P agonists comprising a group of formula X, in treating solid tumors as hereinabove specified, may be demonstrated in animal test methods as well as in clinic, for example in accordance with the methods hereinafter described.

A. In Vitro

A.1 Antitumor Activity

A mouse breast cancer cell line originally isolated from mammary carcinomas is used, e.g. JygMC(A). The cell number is adjusted to $5x10^5$ for plating in fresh medium before the procedure. Cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12 h and then washed twice with PBS, followed by addition of

fresh medium with 10% FCS and additionally incubated for another 12h. Thereafter the cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12h. To release the cells from the block, the cells are washed twice with PBS and replated in fresh medium with 10% FCS. After synchronisation, the cells are incubated with or without various concentrations of a compound of formula I for 3, 6, 9, 12, 18 or 24h. The cells are harvested after treatment with 0.2% EDTA, fixed with ice-cold 70% ethanol solution, hydrolyzed with 250µg/ml of RNaseA (type 1-A: Sigma Chem. Co.) at 37°C for 30 mn and stained with propidium iodide at 10mg/ml for 20 mn. After the incubation period, the number of cells is determined both by counting cells in a Coulter counter and by the SRB colorimetric assay. Under these conditions an S1P agonist, e.g. Compound B in hydrochloride salt form, inhibits the proliferation of the tumor cells at concentrations ranging from 10^{-12} to 10^{-6} M.

A.2 S1P-Mediated HUVEC Tube Formation Assay

For the tube formation assay, HUVEC from passage 2-8 are used and are never greater than 70% confluent before harvesting. Cells are prepared for the assay by washing with Herpes Balanced Saline Solution (HBSS from Clonetics) and then trypsinizing with Trypsin/EDTA (0.25 mg/ml, from Clonetics). After approximately 90 % of the cells have lifted off the plate, an equal volume of Trypsin Neutralizing Solution (TNS from Clonetics) is added and the cells are collected into a conical tube containing at least 10 ml of EBM-2 (Clonetics) + 0.1 % BSA (Sigma) media. Cells are centrifuged at 1000 rpm for 5 minutes and the supernatant is removed and replaced with 5 ml of fresh EBM-2 + 0.1 % BSA. Cells are counted using a hemacytometer and the volume of the cell suspension is adjusted to achieve a concentration of 500,000 cells/ml. Conical tubes are prepared with test compounds at 100 nM, and pertussin toxin (PTx) at 10 ng/ml in each, then 1 ml of the cell suspension is added to each tube. Tubes are then incubated for ½ hour at 37 ºC, 5 % CO_2. The migration assay is performed using Fluoro-Blok 24-Multiwell Insert Plates coated with fibronectin (8 µm pore size, Falcon #351147) instead of the individual inserts in a 24-well plate. Cells and test compounds are prepared and pre-incubated as described above, then 100 µl is added to each approriate well in the Insert Plate. 300 µl of the EBM-2 + 2 % charcoal-stripped media without S1P is added to the bottoms of the wells marked for no stimulation (-), and 300 µl of the media containing S1P (500 nM) is added to the bottoms of the wells marked for stimulation (+). The plate is then incubated for 4 hours at 37 ºC, 5 % CO_2.

Calcein AM, 50 µg/vial, (Molecular Probes #C3100) is prepared by first adding 20 µl DMSO to the vial. Then 12.5 ml of HBSS (per plate) is warmed to 37 ºC and 150 µl is added to the

vial. The contents of the vial are then transferred back to the remaining HBSS to make the final concentration 4 µg/ml Calcein AM.

The Fluoro-Blok plate is removed from the incubator and the top insert plate is separated and "flicked" to remove excess media clinging to the inserts. The insert plate is then transferred to a fresh 24-well plate containing 500 µl/well of the 4 µg/ml Calcein AM. The plate is then incubated for 1½ hours at 37 ºC, 5 % CO_2.

After incubation, the plate is read on a Cytofluor II at an excitation of 485 nm and emission of 530 nm. The Fluoro-Blok coating in the inserts allows only the cells that have migrated to the bottom to be counted. Data are transferred to Excel for calculations, graphs are created using SigmaPlot, and SigmaStat is used for significance tests (t-test). (Figure 7).

Tube formation is quantitated by counting the number of branching points (two independent cords connecting) in 3 independent fields at 4x magnification. The results are reported as follows:

Treatment	Branching Points
PBS	8 ± 5
S1P	42 ± 13
FTY720-Phosphate	48 ± 15
FTY720-Phosphate + S1P	14 ± 7
Compound C-Phosphate	44 ± 16
Compound C-Phosphate + S1P	18 ± 6

These results demonstrate the unique ability of FTY720-Phosphate or Compound C-Phosphate to act as an agonist of angiogenesis on its own, but then surprisingly, as an antagonist of S1P-mediated angiogenesis. Compound C-Phosphate is preferably the racemate or the R-enantiomer. PTx is used as a control to inhibit Giα (EDG-1) mediated activity.

B. In Vivo

B.1 Antitumor Activity

Antitumor activity is expressed as T/C% (mean increase in tumor volumes of treated animals divided by the mean increase of tumor volumes of control animals multiplied by 100).

Aliquots of cancer cells ($1x10^7$), e.g. human A375 melanoma cells, are transplanted into BALB/c-*nu/nu* mice. When the tumors have reached ca. 10x10 mm in size, the animals are assigned randomly to four subgroups and the treatment with a compound of formula I is initiated. Animals are sacrificed after 2 week treatment, at which times tumors and tissues are harvested and prepared for morphological and molecular analysis. The size of the tumors is determined with a caliper. In this assay, an S1P agonist, e.g. Compound B or C (in the hydrochloride salt form), slows tumor growth when administered at a dose of from 0.5 to 5 mg/kg vs saline control: for example, Compound C-HCl when administered at a dose of 2.5mg/kg 5x/week results in a final T/C value of 30%.

B.2 Combination with a VEGF-R protein tyrosin kinase inhibitor

Nude mice transplanted with human MDA-MB-435 breast tumors are treated for 2 weeks with a VEGF-R protein tyrosin kinase inhibitor, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine succinate, at a dose of 100 mg/kg p.o. 5x/week, a S1P receptor agonist, e.g. Compound C (hydrochloride salt), at a dose of 2.5 mg/kg i.v. 5x/week, or a combination of both. Antitumor is expressed as T/C% as indicated above. A combination of Compound C-HCl with 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine succinate produces a greater antitumor effect (T/C% 27) as compared to either agent alone (Compound C-HCl, T/C 66%; 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine succinate, T/C% 91).
Good antitumor responses are also obtained when nude mice are transplanted with human A375 melanoma cells and treated in a similar way with the same combination: the combined treatment results in a T/C% 15 whereas treatment with each agent alone results in a T/C% 35 and 44, respectively.

B.3 Antiangiogenic Activity

Porous chambers containing (i) sphingosine-1-phosphate (5 µM/chamber) or (ii) human VEGF (1 µg/chamber) in 0.5 ml of 0.8% w/v agar (containing heparin, 20 U/ml) are implanted subcutaneously in the flank of mice. S1P or VEGF induces the growth of vascularized tissue around the chamber. This response is dose-dependent and can be quantified by measuring the weight and blood content of the tissue. Mice are treated once a day (i) orally with Compound A (0.3, 3, 30 or 50 mg/kg) or (ii) intravenously with the R enantiomer of Compound C (2.5 mg/kg) or (iii) intravenously with the S enantiomer of Compound C (2.5 mg/kg) or (iv) orally or intravenously with vehicle (5% glucose, 10 ml/kg), starting 4-6 hours before implantation of the chambers and continuing for 4 days. The animals are sacrificed for measurement of the vascularized tissues 24 h after the last dose. The weight and blood content of the vascularized tissues around the chamber is determined.

Animals treated with Compound A or with the R or S enantiomer of Compound C show reduced weight and/or blood content of the vascularized tissues compared to animals treated with vehicle alone.

C. Clinical Trial

C.1 Investigation of clinical benefit of a S1P receptor agonist, e.g. a compound of formula I, II or III, e.g. Compound A, B or C

20 patients with progressing, advanced-stage solid tumors, resistant or refractory to standard therapies, to receive said compound at a dosage as determined by a dose escalating study. The general clinical state of the patient is investigated weekly by physical and laboratory examination. Changes in tumor and metastases burden are assessed every 2 months by radiological examination. Initially patients receive treatment for 2 months. Thereafter, they remain on treatment for as long as their disease does not progress and the drug is satisfactorily tolerated.

Main variables for evaluation: Safety (adverse events), standard serum biochemistry and haematology, tumor dimensions by computerised tomographic (CT) scan or magnetic resonance imaging (MRI).

C.2 Combined Treatment

Suitable clinical studies are, for example, open label non-randomized, dose escalation studies in patients with advanced solid tumors. Such studies prove in particular the synergism of the active ingredients of the combination of the invention. The beneficial effects on proliferative diseases can be determined directly through the results of these studies or by changes in the study design which are known as such to a person skilled in the art. Such studies are, in particular, suitable to compare the effects of a monotherapy using the active ingredients and a combination of the invention. Preferably, the dose of agent (a) is escalated until the Maximum Tolerated Dosage is reached, and the co-agent (b) is administered with a fixed dose. Alternatively, the agent (a) is administered in a fixed dose and the dose of co-agent (b) is escalated. Each patient receives doses of the agent (a) either daily or intermittent. The efficacy of the treatment can be determined in such studies, e.g., after 12, 18 or 24 weeks by radiologic evaluation of the tumors every 6 weeks.

Alternatively, a placebo-controlled, double blind study can be used in order to prove the benefits of the combination of the invention mentioned herein.

Daily dosages required in practicing the method of the present invention when a S1P receptor agonist alone is used will vary depending upon, for example, the compound used, the host, the mode of administration and the severity of the condition to be treated. A preferred daily dosage range is about from 0.1 to 100 mg as a single dose or in divided

doses. Suitable daily dosages for patients are on the order of from e.g. 0.1 to 50 mg p.o. The S1P receptor agonist may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions, nasally, pulmonary (by inhalation) or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration comprise from ca. 0.1 to 30 mg, usually 0.25 to 30 mg S1P receptor agonist, together with one or more pharmaceutically acceptable diluents or carriers therefore. In order to inhibit angiogenesis it is important to select a sufficiently high dose of the S1P receptor agonist, as low concentrations of S1P receptor agonists promote angiogenesis. A suitable dose for providing an anti-angiogenic effect when a S1P agonist is administered to a patient may be selected by concentration- and dose-escalating studies as described at A, B, and C above.

The combination of the invention can also be applied in combination with surgical intervention, mild prolonged whole body hyperthermia and/or irradiation therapy.

The administration of a pharmaceutical combination of the invention results in a beneficial effect, e.g. a synergistic therapeutic effect, e.g. with regard to slowing down, arresting or reversing the neoplasm formation, metastases spread or growth or a longer duration of tumor response or inhibition of angiogenesis; it may also result in other beneficial effects, e.g. less side-effects, an improved quality of life or a decreased mortality and morbidity, compared to a monotherapy applying only one of the pharmaceutically active ingredients used in the combination of the invention, in particular in the treatment of a tumor that is refractory to other chemotherapeutics known as anti-cancer agents.

A further benefit is that lower doses of the active ingredients of the combination of the invention can be used, for example, that the dosages need not only often be smaller but are also applied less frequently, or can be used in order to diminish the incidence of side-effects, while controlling the growth of neoplasm formation. This is in accordance with the desires and requirements of the patients to be treated.

According to one embodiment of the invention, a preferred pharmaceutical combination comprises

a) a compound of formula I, II, III, IVa, IVb, V or VI, e.g. Compound A, B or C, and

b) as a co-agent, one or more compounds as indicated in paragraphs (ii), (iii), (iv), (v), (vii) or (xi) above, e.g. carboplatin, cisplatinum, paclitaxel, docetaxel, gemcitabine, doxorubicin, a compound targeting, decreasing or inhibiting the activity of the vascular endothelial growth factor family of receptor tyrosine kinases (VEGFR) or the platelet-derived growth factor-receptors (PDGFR), a bisphosphonate or a mTOR inhibitor.

A further embodiment of the invention relates to the use of S1P receptor agonist (a) in combination with a chemotherapeutic agent (b) in the treatment of a lymphatic or myeloid cancer, e.g. as disclosed above. The combination may comprise as a further co-agent b) e.g. busulfan, cytarabine, 6-thioguanine, fludarabine, hydroxyurea, procarbazine, bleomycin or methotrexate. Topoisomerase II inhibitors e,g. daunorubicin or, particularly, compounds which target, decrease or inhibit the activity of PDGFR or of c-Abl family members and their gene fusion products, e.g. imatinib, are preferred as co-agent (b), e.g. for use in the treatment of a lymphatic cancer.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

It is one objective of this invention to provide a pharmaceutical composition comprising a quantity, which is jointly therapeutically effective against a proliferative malignant disease comprising a combination of the invention. In this composition, the first agent a) and co-agent (b) can be administered together, one after the other or separately in one combined unit dosage form or in two separate unit dosage forms. The unit dosage form may also be a fixed combination.

The pharmaceutical compositions according to the invention can be prepared in a manner known per se and are those suitable for enteral, such as oral or rectal, and parenteral administration to mammals (warm-blooded animals), including humans, comprising a therapeutically effective amount of at least one pharmacologically active combination partner alone, e.g. as indicated above, or in combination with one or more pharmaceutically acceptable carriers or diluents, especially suitable for enteral or parenteral application.

Suitable pharmaceutical compositions contain, for example, from about 0.1 % to about 99.9%, preferably from about 1 % to about 60 %, of the active ingredient(s). Pharmaceutical preparations for the combination therapy for enteral or parenteral administration are, for example, those in unit dosage forms, such as sugar-coated tablets, tablets, capsules or suppositories, or ampoules. If not indicated otherwise, these are prepared in a manner known per se, for example by means of conventional mixing, granulating, sugar-coating, dissolving or lyophilizing processes. It will be appreciated that the unit content of a combination partner contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount can be reached by administration of a plurality of dosage units.

In particular, a therapeutically effective amount of each of the combination partner of the combination of the invention may be administered simultaneously or sequentially and in any order, and the components may be administered separately or as a fixed combination. For example, the method of delay of progression or treatment of a proliferative malignant disease according to the invention may comprise (i) administration of the first agent a) in free or pharmaceutically acceptable salt form and (ii) administration of a co-agent b) in free or pharmaceutically acceptable salt form, simultaneously or sequentially in any order, in jointly therapeutically effective amounts, preferably in synergistically effective amounts, e.g. in daily or intermittently dosages corresponding to the amounts described herein. The individual combination partners of the combination of the invention may be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. Furthermore, the term administering also encompasses the use of a pro-drug of a combination partner that convert *in vivo* to the combination partner as such. The instant invention is therefore to be understood as embracing all such regimens of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

The effective dosage of each of the combination partners employed in the combination of the invention may vary depending on the particular compound or pharmaceutical composition employed, the mode of administration, the condition being treated, the severity of the condition being treated. Thus, the dosage regimen of the combination of the invention is selected in accordance with a variety of factors including the route of administration and the renal and hepatic function of the patient. A physician, clinician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the single active ingredients required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentration of the active ingredients within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the active ingredients' availability to target sites.

Daily dosages for the first agent or component (a) will, of course, vary depending on a variety of factors, for example the compound chosen, the particular condition to be treated and the desired effect. In general, however, satisfactory results are achieved on administration of a S1P receptor agonist, e.g. Compound A, B or C, at daily dosage rates of the order of ca. 0.1 to 100 mg as a single dose or in divided doses. The S1P receptor agonist may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration

comprise from ca. 0.1 to 30 mg component (a), e.g. 0.1 to 25 mg, together with one or more pharmaceutically acceptable diluents or carriers therefor.

Fadrozole may be administered orally to a human in a dosage range varying from about 0.5 to about 10 mg/day, preferably from about 1 to about 2.5 mg/day. Exemestane may be administered orally to a human in a dosage range varying from about 5 to about 200 mg/day, preferably from about 10 to about 25 mg/day, or parenterally from about 50 to 500 mg/day, preferably from about 100 to about 250 mg/day. If the drug shall be administered in a separate pharmaceutical composition, it can be administered in the form disclosed in GB 2,177,700. Formestane may be administered parenterally to a human in a dosage range varying from about 100 to 500 mg/day, preferably from about 250 to about 300 mg/day. Anastrozole may be administered orallly to a human in a dosage range varying from about 0.25 to 20 mg/day, preferably from about 0.5 to about 2.5 mg/day. Aminogluthemide may be administered to a human in a dosage range varying from about 200 to 500 mg/day.

Tamoxifen citrate may be administered to a human in a dosage range varying from about 10 to 40 mg/day.

Vinblastine may be administered to a human in a dosage range varying from about 1.5 to 10 mg/m^{2}day. Vincristine sulfate may be administered parenterally to a human in a dosage range varying from about 0.025 to 0.05 mg/kg body weight * week. Vinorelbine may be administered to a human in a dosage range varying from about 10 to 50 mg/m^{2}day.

Etoposide phosphate may be administered to a human in a dosage range varying from about 25 to 115 mg/m^{2}day, e.g. 56.8 or 113.6 mg/m^{2}day.

Teniposide may be administered to a human in a dosage range varying from about 75 to 150 mg about every two weeks. Doxorubicin may be administered to a human in a dosage range varying from about 10 to 100 mg/m^{2}day, e.g. 25 or 50 mg/m^{2}day. Epirubicin may be administered to a human in a dosage range varying from about 10 to 200 mg/m^{2}day. Idarubicin may be administered to a human in a dosage range varying from about 0.5 to 50 mg/m^{2}day. Mitoxantrone may be administered to a human in a dosage range varying from about 2.5 to 25 mg/m^{2}day.

Paclitaxel may be administered to a human in a dosage range varying from about 50 to 300 mg/m^{2}day. Docetaxel may be administered to a human in a dosage range varying from about 25 to 100 mg/m^{2}day.

Cyclophosphamide may be administered to a human in a dosage range varying from about 50 to 1500 mg/m^{2}day. Melphalan may be administered to a human in a dosage range varying from about 0.5 to 10 mg/m^{2}day.

5-Fluorouracil may be administered to a human in a dosage range varying from about 50 to 1000 mg/m^{2}day, e.g. 500 mg/m^{2}day. Capecitabine may be administered to a human in a dosage range varying from about 10 to 1000 mg/m^{2}day. Gemcitabine hydrochloride may be administered to a human in a dosage range varying from about 1000 mg/m^2/week. Methotrexate may be administered to a human in a dosage range varying from about 5 to 500 mg/m^{2}day.

Topotecan may be administered to a human in a dosage range varying from about 1 to 5 mg/m^{2}day. Irinotecan may be administered to a human in a dosage range varying from about 50 to 350 mg/m^{2}day.

Carboplatin may be administered to a human in a dosage range varying from about 200 to 400 mg/m^2 about every four weeks. Cisplatin may be administered to a human in a dosage range varying from about 25 to 75 mg/m^2 about every three weeks. Oxaliplatin may be administered to a human in a dosage range varying from about 50 to 85 mg/m^2 every two weeks.

Imatinib may be administered to a human in a dosage in the range of about 2.5 to 850 mg/day, more preferably 5 to 600 mg/day and most preferably 20 to 300 mg/day.

Alendronic acid may be administered to a human in a dosage range varying from about 5 to 10 mg/day. Clodronic acid may be administered to a human e.g. in a dosage range varying from about 750 to 1500 mg/day. Etridonic acid may be administered to a human in a dosage range varying from about 200 to 400 mg/day. Ibandronic acid may be administered to a human in a dosage range varying from about 1 to 4 mg every three to four weeks. Risedronic acid may be administered to a human in a dosage range varying from about 20 to 30 mg/day. Pamidronic acid may be administered to a human in a dosage range varying from about 15 to 90 mg every three to four weeks. Tiludronic acid may be administered to a human in a dosage range varying from about 200 to 400 mg/day.

Trastuzumab may be administered to a human in a dosage range varying from about 1 to 4 mg/m^2/week.

Bicalutamide may be administered to a human in a dosage range varying from about 25 to 50 mg/m^{2}day.

1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or salt thereof, e.g. succinate, may be administered to a human in a dosage range of about 50 to 1500, more preferably about 100 to 750, and most preferably 250 to 500, mg/day.

Rapamycin or a derivative thereof, e.g. 40-O-(2-hydroxyethyl)-rapamycin, may be administered in a dosage range varying from about 0.1 to 25 mg.

Formulation Example: soft capsules

Compound of formula I,	
e.g. Compound A, HCl	30 mg
Polyethylene glycol 300	300 mg
Polysorbate 80	20 mg
Total	350 mg

The S1P receptor agonists, e.g. a S1P receptor agonist comprising a group of formula X, are well tolerated at dosages required for use in accordance with the present invention. For example, the acute LD_{50} for Compound A is > 10 mg/kg p.o. in rats and monkeys.

In a further aspect, the present invention relates to the use of S1P agonists as pro-angiogenic drugs. Induction of neo-angiogenesis has lately been recognized as an excellent target in a number of conditions (e.g.myocardial angiogenesis, wound healing or diabetic vascular dysfunction/vasculopathy).

As described above, high concentrations of S1P receptor agonists (2 μM or greater, e.g. 2-5 μM or around 5 μM) exhibit anti-angiogenic effects, and S1P receptor agonists can inhibit VEGF-induced angiogenesis. In contrast, low concentrations (0.1 -1 μM, e.g. 0.1 – 0.5 μM or 0.5 – 1 μM) of S1P agonists have an enhancing effect on angiogenesis and are able to potentiate VEGF-mediated angiogenesis. Thus, S1P agonists may have biphasic effects in angiogenesis.

Accordingly, the present invention further provides:

8. Use of a S1P agonist, e.g. a S1P agonist comprising a group of formula X, e.g. Compound A or Compound A-phosphate, in the induction of the neo-angiogenesis process,

e.g. as a pro-angionenic agent, e.g. in indications where a promotion of angiogenesis is indicated;

9. A process for the preparation of a medicament for the treatment or prevention of diseases mediated by the inhibition of the neo-angiogenesis process, e.g. mediated by anti-angionenic factors, e.g. in indications where a promotion of angiogenesis is indicated, e.g. in wound healing or in the treatment of myocardial infarction or diabetic vascular dysfunction/vasculopathy, comprising using a S1P receptor agonist, e.g. a S1P agonist comprising a group of formula X, e.g. Compound A or Compound A-phosphate, as an active ingredient;

10. A method of treating or preventing diseases mediated by the inhibition of the neo-angiogenesis process, e.g. mediated by anti-angionenic factors, e.g. in indications where a promotion of angiogenesis is indicated, such as e.g. in wound healing or in the treatment of myocardial infarction or diabetic vascular dysfunction/vasculopathy, comprising administering an effective amount of a S1P receptor agonist, e.g. a S1P agonist comprising a group of formula X, e.g. Compound A or Compound A-phosphate, to a subject in need of such treatment.

S1P agonists suitable for promoting angiogenesis include those defined above in relation to the treatment of cancer, e.g. S1P agonists comprising a group of formula X or compounds according to formulae I to IX, or pharmaceutically acceptable salts or esters thereof. Preferably the S1P agonist is Compound A-phosphate. The S1P agonist may be used alone, or in combination with one or more further agents which promote angiogenesis, e.g. VEGF.

In order to promote angiogenesis it is important to select a sufficiently low dose of the S1P receptor agonist, as high concentrations of S1P receptor agonists inhibit angiogenesis. A suitable dose for providing a pro-angiogenic effect when a S1P agonist is administered to a patient may be selected by concentration- and dose-escalating studies as described at A, B, and C above.

Description of the Figures

Figure 1

shows that Compound A-phosphate strongly promotes capillary-like network formation in a bell-shape dose-dependent manner showing maximal activity around 0.5 µM.

Figure 2

shows that both Compound A-phosphate and Compound A at 0.5 - 1 µM do not attenuate VEGF-mediated remodelling but rather cooperate with polypeptide growth factor.

Figure 3

Shows that Compound A-phosphate as well as S1P-stimulated tube formation is practically completely inhibited by pertussis toxin (PTX, 50 ng/ml), an inhibitor of heterotrimeric G proteins of $\alpha_{i/o}$-type. This may be interpreted as a possible involvement of EDG-1 ($S1P_1$) receptor-mediated signaling events in Compound A-phosphate-stimulated bioresponses.

Figure 4

Shows, that sphingosine at 1 µM, which itself seems to be less potent than S1P, attenuates the ability of both S1P and Compound A-phosphate to induce capillary-like structures, without having an inhibitory effect on the VEGF-induced tube formation. In this respect, sphingosine behaves different from Compound A. The data indicate that the balance between sphingosine and S1P seems to be critically important for endothelial cell activation/angiogenesis most likely via the EDG receptor family. Importantly, high concentrations of sphingosine and Compound A (2 – 5 µM) inhibits VEGF-triggered tube formation.

Figure 5

Shows that the treatment of HUVEC with Compound A-phosphate at 0.5 µM may result in transient activation of ERK1/2 with a peak of phosphorylation/activation at 10 minutes and returning to baseline by 20 minutes

Figure 6

It was tested whether Compound A, Compound A-phosphate, sphingosine or S1P also do induce tissue factor on HUVEC. The data found demonstrate that none of these compounds alone or in combinations may elevate tissue factor activity as shown in Figure 6. Compound A and Compound A-phosphate may slightly enhance the VEGF- but not TNF-α-induced tissue factor.

Figure 7

Shows the effect of Compound C in a S1P-mediated HUVEC tube formation assay.

The following abbreviations are used:

BSA: bovine serum albumine

ECGS: endothelial cell growth factor set

ECL: enhanced chemiluminescence

S: sphingosine

PBS: phosphate-buffered saline

JNK1/2: c-jun-N-terminal kinase1/2

RT: room temperature

TF equivalents: tissue factor equivalents

EGR-1/NFAT: early growth response protein 1/nuclear factor of activated T-cells

F1P: Compound A-phosphate (FTY720-phosphate)

Utility of the S1P receptor agonists, e.g. the S1P agonists comprising a group of formula X, in the promotion of angiogenesis may be demonstrated for example in accordance with the methods described hereinafter.

D. Cell culture and Materials

Human umbilical vein endothelial cells (HUVEC) are cultured at 37°C and 5% CO_2 in medium M199 supplemented with 20% SCS (HyClone, Logan, UT), 1U/ml heparin, 50 µg/ml ECGS, 2 mM glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin. Cells are used for experiments up to passage number 5. Short-starved HUVEC are obtained by starving with 1% SCS-containing M199 for 5 h. Recombinant human $VEGF_{165}$ is obtained from PromoCell (Heidelberg, Germany). Phospho-specific ERK1/2, p38 kinase polyclonal antibodies, nonphospho ERK1/2 antibodies and LumiGLO chemiluminescent reagent are from New England BioLabs (Beverly, MA), polyclonal IκB antibodies from Santa Cruz Biotechnology (Santa Cruz, Calif.). Peroxidase-conjugated donkey anti-rabbit immunoglobulin G (IgG) and sheep anti-mouse IgG are purchased from Amersham LIFE SCIENCE (Amersham Place, England). Immobilon-P transfer membranes are products of Millipore (Bedford, MA). S is obtained from Sigma Chemical Co.; S1P is from Biomol. Compound A-phosphate stock solution is prepared by the following protocol. Compound A-phosphate is dissolved in methanol tracing with concentrated HCl (0.5 mg Compound A-phosphate in 500 µl of methanol plus 2 µl of HCl). Solvent from the resulting solution is evaporated under vacuum and the residue obtained is redissolved (variant 1) in 0.1 % of defatted BSA solution in sterile deionized water (500 µl) or (variant 2) in 0.5 % Triton X-100 in deionized water. The resulting stock solutions (2.5 mM) are sonicated and stored at 4°C.

Clotting Assay

Cells are seeded in 6-well plates at 80-90% confluency and grown overnight. Cells are scraped from the plates and analyzed for tissue factor activity according to the method as described in Clauss, M., J. Biol. Chem. 271,17629-17634 (1996), Mechtcheriakova, D., Blood 93,3811-3823 (1999). Briefly, after induction for 4 hours with VEGF (1.5 nM), TNF-α (100 U/ml), S (0.5-2 µM), S1P (0.5-2 µM), Compound A (0.5-2 µM), and Compound A-phosphate (0.5-2 µM), cells are washed twice and then scraped in 1ml clotting buffer (12 mM sodium acetate, 7 mM diethylbarbitate and 130 mM sodium chloride; pH 7.4). 50 µl of resuspended cells are mixed with 50 µl of citrated plasma, and clotting times are determined after recalcification with 50 µl of 20 mM $CaCl_2$ solution at 37°C. TF-equivalents are determined by using a standard curve obtained from rabbit brain thromboplastin.

E. Western Blot Analysis

After various treatments, the cells are washed twice with cold PBS, lysed in 100 µl of Laemmli buffer, scraped and heated for 5 min at 95°C. Total cell lysates are separated by SDS-PAGE and transferred to Immobilon-P membrane. The membrane is blocked for 30 minutes with PBS containing 0.1% Tween-20 and 3% skim milk and incubated for 1 hour at RT with a primary antibody diluted in blocking buffer. The membrane obtained is washed three times for 5 minutes with PBS containing 0.1% Tween-20 and incubated with peroxidase-conjugated secondary antibody for 1 hour at RT. After a washing step, the membrane is incubated for 1 minute with ECL reagent and exposed to film as required. For reprobing with another antibody, the membrane is washed twice in PBS, stripped for 30 min at 55°C with stripping buffer (62.5 mM Tris-HCL, pH 6.8, 2% SDS, 100 mM 2-mercaptoethanol) and washed three times for 5 minutes with PBS at RT. The membrane is stored wet wrapped in SaranWrap at 4°C after each immunodetection.

In vitro angiogenesis assay on Matrigel

Morphogenesis of endothelial cells into capillary-like structures on Growth Factor Reduced Matrigel Matrix (BD Bioscience) is performed according to the manufacture procedure. Briefly, HUVEC are trypsinized, resuspended in serum-free M199 medium containing soybean trypsin inhibitor (1mg/ml, Sigma). After centrifugation cells are resuspended in serum-free medium at a density 0.5×10^5 cells/ml, and cell suspension is seeded in 96-well cell culture plates (Costar, Corning Incorporated) precoated with 50 µl of Matrigel in the absence or presence of various stimuli: VEGF at 1.5 nM, S1P at 0.1-2 µM, S at 0.5-2 µM, Compound A at 0.5-2 µM, and Compound A-phosphate at 0.1-2 µM. Eight hours later, cells

on Matrigel are fixed with 3 % formaldehyde in PBS and kept at 4°C. Results are quantitated from images made with a Nikon Diaphot microscope equipped with a cooled CCD camera (Kappa GmbH, Gleichen, Germany) by direct counting of branching points on two microscopic fields from each well done in duplicates.

F. Compound A-phosphate-induces morphogenesis of endothelial cells in in vitro tube formation assay on Matrigel and a possible involvment of G_i-mediated signaling pathway(s)

The effect of Compound A and Compound A-phosphate on morphogenic differentiation of endothelial cells is determined using an in vitro angiogenesis assay on Matrigel. Endothelial cell morphogenesis is a complex process that requires cell-extracellular matrix interactions, followed by matrix remodelling, stimulated migration, cell-cell interactions, and perivascular proteolysis. As shown in Figure 1, Compound A-phosphate strongly may promote capillary-like network formation in a bell-shape dose-dependent manner showing maximal activity around 0.5 µM. The number of branching points per microscopic field, which reflects the induction potency of the stimulus, is comparable for Compound A-phosphate and S1P, and may exceed significantly the VEGF-triggered effects. Compound A itself at 0.5 - 1 µM has a weak, in comparison to Compound A-phosphate, but consistent enhancing effect. Both Compound A-phosphate and Compound A at 0.5 - 1 µM does not attenuate VEGF-mediated remodelling but rather cooperates with polypeptide growth factor (see e.g. Figure 2). Furthermore, Compound A-phosphate- as well as S1P-stimulated tube formation is completely inhibited by pertussis toxin (PTX, 50 ng/ml), an inhibitor of heterotrimeric G proteins of $\alpha_{i/o}$-type. This may be interpreted as a possible involvement of EDG-1 ($S1P_1$) receptor-mediated signaling events in Compound A-phosphate-stimulated bioresponses (see e.g. Figure 3). S at 1 µM, which itself seems to be less potent than S1P, attenuates the ability of both S1P and Compound A-phosphate to induce capillary-like structures, without having an inhibitory effect on the VEGF-induced tube formation (see e.g. Figure 4). In this respect, S behaves differently from Compound A. The data indicate that the balance between S and S1P seems to be critically important for endothelial cell activation/angiogenesis most likely via the EDG receptor family. Importantly, high concentrations of S and Compound A (2 – 5 µM) inhibited VEGF-triggered tube formation. That data suggest biphasic dose-dependent effects of Compound A and Compound A-phosphate on angiogenesis in vitro.

G. Activation of ERK1/2 MAP kinases by Compound A-phosphate

Signal transduction via MAP kinases plays a key role in a variety of endothelial cell functions. Treatment of HUVEC with Compound A-phosphate at 0.5 µM may result in transient activation of ERK1/2 with a peak of phosphorylation/activation at 10 minutes and returning to baseline by 20 minutes (see e.g. Figure 5). No activation of p38 kinase and JNK1/2 by Compound A-phosphate is detectable in HUVEC. Furthermore, Compound A-phosphate may trigger ERK1/2 activation in a dose-dependent manner, showing stronger activity at 2 µM. This is in contrast to the results from the tube formation assay, where Compound A-phosphate at 2 µM may be less potent than at 0.5 µM. Neither Compound A nor S are able to induce MAP kinase activation in endothelial cells in a kinetics ranging from 5 minute to 60 minute treatment. To estimate the possible role of inflammatory/NFκB-dependent program in Compound A-phosphate-stimulated bioresponses of endothelial cells, the membranes are reprobed with anti-IκB antibodies. IκB levels are not affected by Compound A-phosphate treatment. Moreover the treatment of endothelial cells with Compound A-phosphate may fail to induce E-Selectin expression as a NFκB-dependent secondary responsive gene. Thus, the data strongly indicate that Compound A-phosphate signaling does not involve NFκB activation – the main cascade in the acute inflammatory response in endothelial cells.

H. Compound A and Compound A-phosphate do not induce tissue factor expression on endothelial cells

An important characteristic feature of both classical inflammatory stimulus TNF-α and the main angiogenic growth factor VEGF on endothelial cells is their potency to upregulate tissue factor. Compound A, Compound A-phosphate, S or S1P are tested whether they also do induce tissue factor on HUVEC. The data found demonstrate that none of these compounds alone or in combinations may elevate tissue factor activity (see e.g. Figure 6). Compound A and Compound A-phosphate may slightly enhance the VEGF- but not TNF-α-induced tissue factor. The data obtained together indicate that Compound A, Compound A-phosphate, S and S1P mechanistically work distinctly to angiogenic VEGF and inflammatory TNF-α.

I. Binding affinity of S1P receptor agonists to individual human S1P receptors may be determined in following assays:

Transient transfection of human S1P receptors into HEK293 cells

EDG receptors and G_i proteins are cloned, and equal amounts of 4 cDNAs for the EDG receptor, G_i-α, G_i-β and G_i-γ are mixed and used to transfect monolayers of HEK293 cells using the calcium phosphate precipitate method (M. Wigler et al., Cell. 1977;11;223 and DS.

Im et al., Mol. Pharmacol. 2000;57;753). Briefly, a DNA mixture containing 25 µg of DNA and 0.25 M CaCl is added to HEPES-buffered 2 mM Na_2HPO_4. Subconfluent monolayers of HEK293 cells are poisoned with 25 mM chloroquine, and the DNA precipitate is then applied to the cells. After 4 h, the monolayers are washed with phosphate-buffered saline and refed media (90% 1:1 Dulbecco's modified essential media (DMEM):F-12 + 10% fetal bovine serum). The cells are harvested 48-72 h after addition of the DNA by scraping in HME buffer (in mM: 20 HEPES, 5 $MgCl_2$, 1 EDTA, pH 7.4) containing 10% sucrose on ice, and disrupted using a Dounce homogenizer. After centrifugation at 800xg, the supernatant is diluted with HME without sucrose and centrifuged at 100,000xg for 1h. The resulting pellet is rehomogenized and centrifuged a second hour at 100,000xg. This crude membrane pellet is resuspended in HME with sucrose, aliquoted, and snap-frozen by immersion in liquid nitrogen. The membranes are stored at 70°C. Protein concentration is determined spectroscopically by Bradford protein assay.

GTPγS binding assay using S1P receptor/HEK293 membrane preparations

GTPγS binding experiments are performed as described by DS. Im et al., Mol. Pharmacol. 2000; 57:753. Ligand-mediated GTPγS binding to G-proteins is measured in GTP binding buffer (in mM: 50 HEPES, 100 NaCl, 10 $MgCl_2$, pH 7.5) using 25 µg of a membrane preparation from transiently transfected HEK293 cells. Ligand is added to membranes in the presence of 10 µM GDP and 0.1 nM [^{35}S]GTPγS (1200 Ci/mmol) and incubated at 30°C for 30 min. Bound GTPγS is separated from unbound using the Brandel harvester (Gaithersburg, MD) and counted with a liquid scintillation counter.

Claims

1. A method for treating solid tumors or inhibiting growth of solid tumors in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist, with the proviso that when the S1P receptor agonist is FTY720 or FTY720-phosphate, the tumor is other than breast, prostate, bladder, kidney or lung tumor.

2. A method for treating solid tumor invasiveness or symptoms associated with such tumor growth, preventing metastatic spread of tumours or for preventing or inhibiting growth of micrometastasis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist.

3. A method for inhibiting or controlling deregulated angiogenesis, e.g. sphingosine-1-phosphate (S1P) mediated angiogenesis, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist.

4. A method for preventing or treating diseases mediated by a neo-angiogenesis process or associated with deregulated angiogenesis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist.

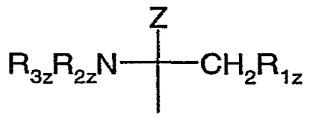
5. A method for enhancing the activity of a chemotherapeutic agent or for overcoming resistance to a chemotherapeutic agent in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist, either concomitantly or sequentially with said chemotherapeutic agent.

6. A method according to any preceding claim wherein the S1P receptor agonist is administered intermittently.

7. A method according to any preceding claim comprising co-administration, concomitantly or in sequence, of a therapeutically effective amount of a S1P receptor agonist and a second drug substance, said second drug substance being a chemotherapeutic agent.

8. A method for treating lymphoproliferative or myeloproliferative disorders comprising co-administering to said subject, concomitantly or in sequence, of a S1P receptor agonist, and a second drug substance, said second drug substance being a chemotherapeutic agent.

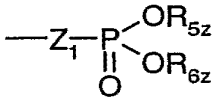
9. A method according to any preceding claim, wherein the S1P receptor agonist comprises a group of formula X:



X

wherein

Z is H; C_{1-6}alkyl; C_{2-6}alkenyl; C_{2-6}alkynyl; phenyl; phenyl substituted by OH; C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH; or CH_2-R_{4z} wherein R_{4z} is OH, acyloxy or a residue of formula (a)



(a)

wherein Z_1 is a direct bond or O, preferably O; each of R_{5z} and R_{6z}, independently, is H, or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms;

R_{1z} is OH, acyloxy or a residue of formula (a); and each of R_{2z} and R_{3z}, independently, is H, C_{1-4}alkyl or acyl.

10. A pharmaceutical combination comprising a) a first agent which is a S1P receptor agonist and b) a co-agent which is a chemotherapeutic agent.

11. A combination according to claim 10, wherein the co-agent is selected from

i. an aromatase inhibitor,
ii. an antiestrogen, an anti-androgen or a gonadorelin agonist,
iii. a topoisomerase I inhibitor or a topoisomerase II inhibitor,
iv. a microtubule active agent, an alkylating agent, an antineoplastic antimetabolite or a platin compound,
v. a compound targeting/decreasing a protein or lipid kinase activity or a protein or lipid phosphatase activity, a further anti-angiogenic compound or a compound which induces cell differentiation processes,
vi. a bradykinin 1 receptor or an angiotensin II antagonist,
vii. a cyclooxygenase inhibitor, a bisphosphonate, a histone deacetylase inhibitor, a heparanase inhibitor, a biological response modifier, an ubiquitination inhibitor, or an inhibitor which blocks anti-apoptotic pathways,
viii. an inhibitor of Ras oncogenic isoforms,
ix. a telomerase inhibitor,

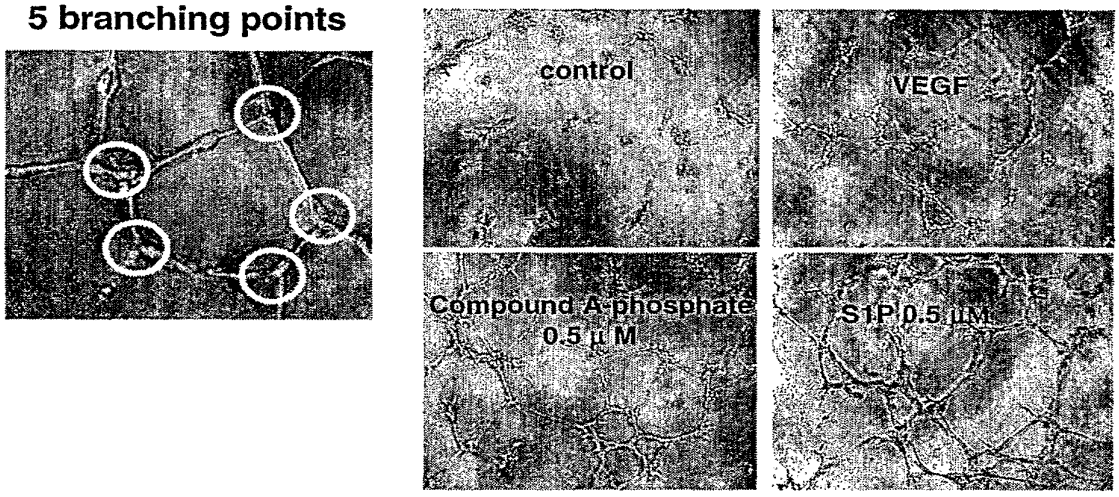
x. a protease inhibitor, a matrix metalloproteinase inhibitor, a methionine aminopeptidase inhibitor, or a proteosome inhibitor, and/or

xi) a mTOR inhibitor.

12. A method of treating or preventing diseases mediated by inhibition of the neo-angiogenesis process comprising administering an effective amount of an S1P receptor agonist to a subject in need of such treatment.

13. A method according to claim 12, wherein the S1P receptor agonist comprises a group of formula X as defined in claim 9.

Fig 1


5 branching points
control
VEGF
Compound A-phosphate 0.5 µM
S1P 0.5 µM

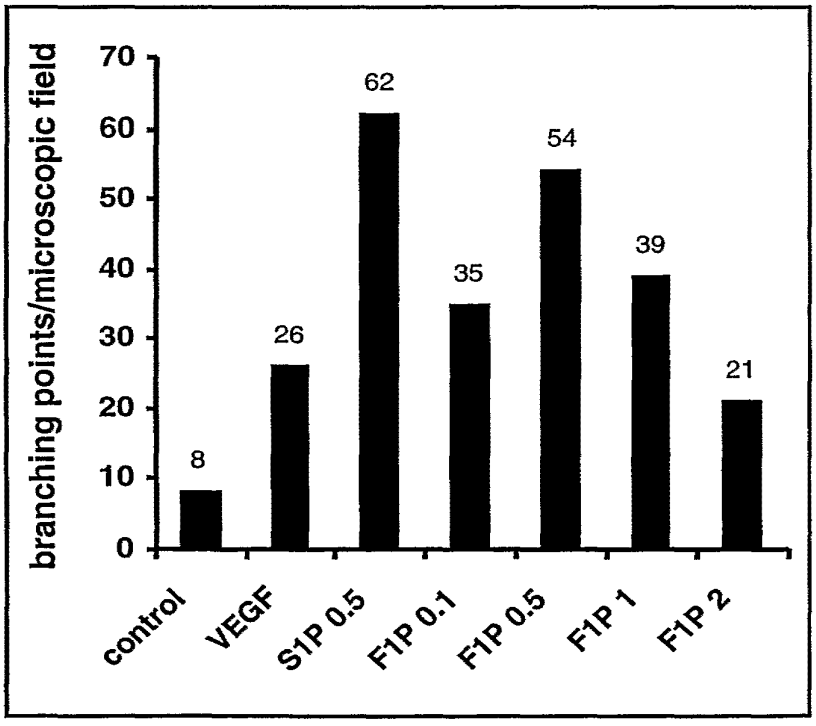

branching points/microscopic field
0
10
20
30
40
50
60
70
8
26
62
35
54
39
21
control
VEGF
S1P 0.5
F1P 0.1
F1P 0.5
F1P 1
F1P 2

Fig. 2

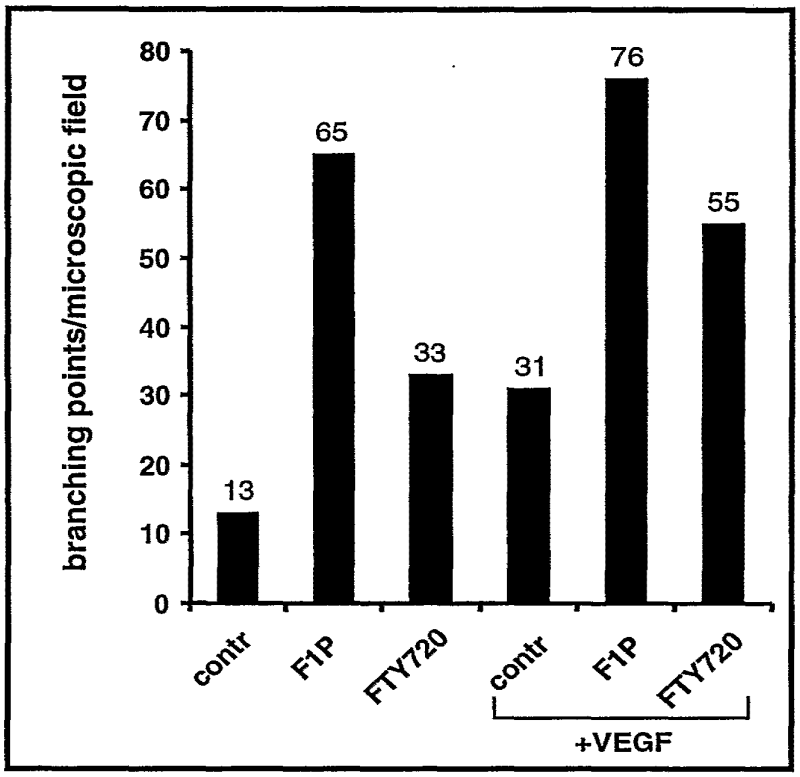

branching points/microscopic field
80
70
60
50
40
30
20
10
0
13
65
33
31
76
55
contr
F1P
FTY720
contr
F1P
FTY720
+VEGF

Fig 3

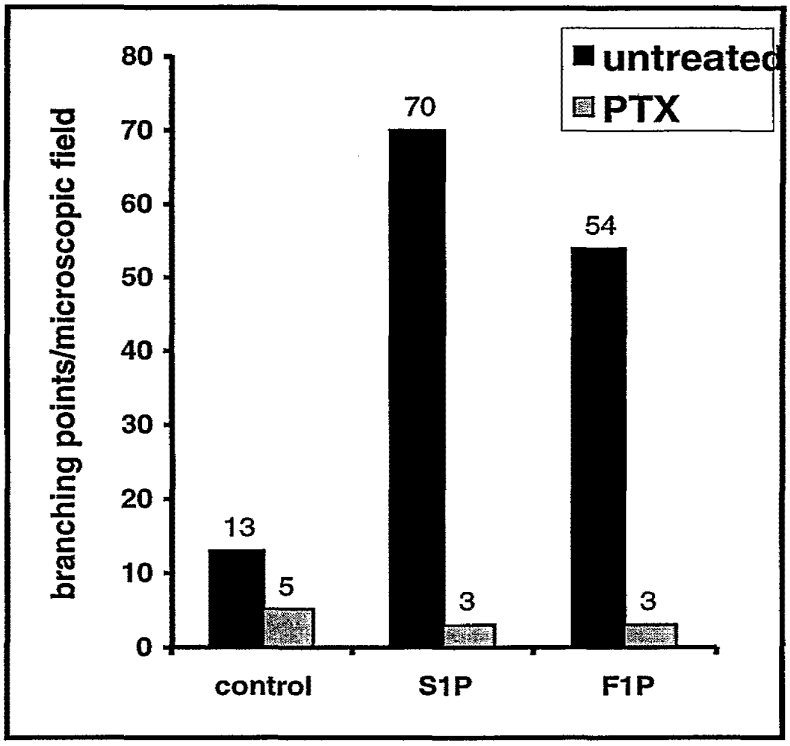

branching points/microscopic field
80
70
60
50
40
30
20
10
0
untreated
PTX
13
5
70
3
54
3
control
S1P
F1P

Fig 4

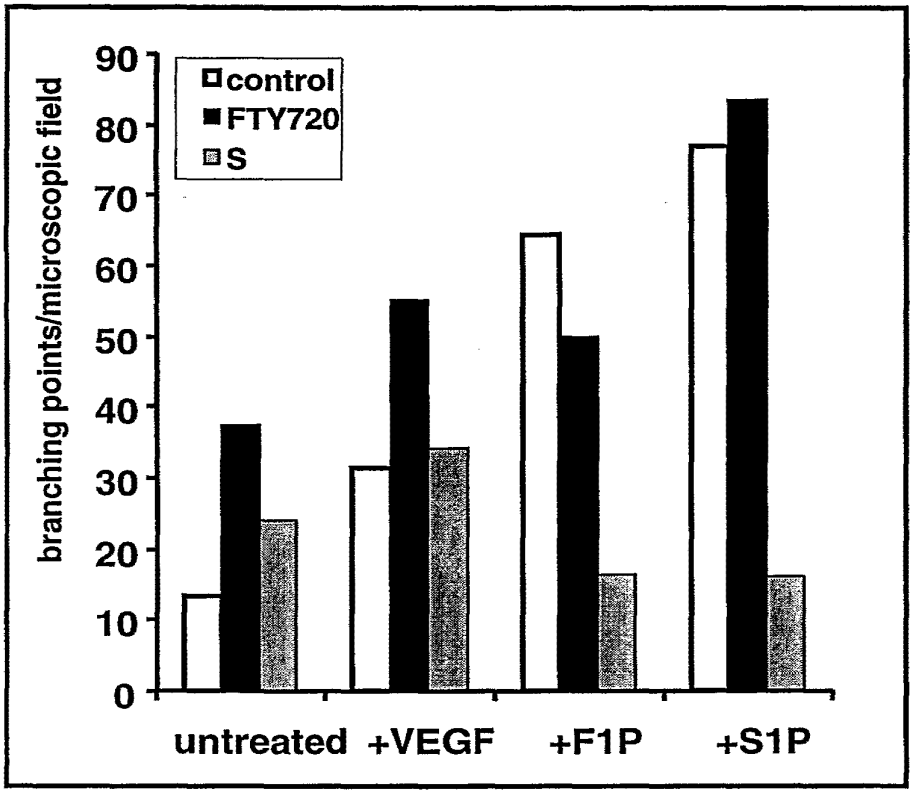

branching points/microscopic field
90
80
70
60
50
40
30
20
10
0
control
FTY720
S
untreated
+VEGF
+F1P
+S1P

Fig 5

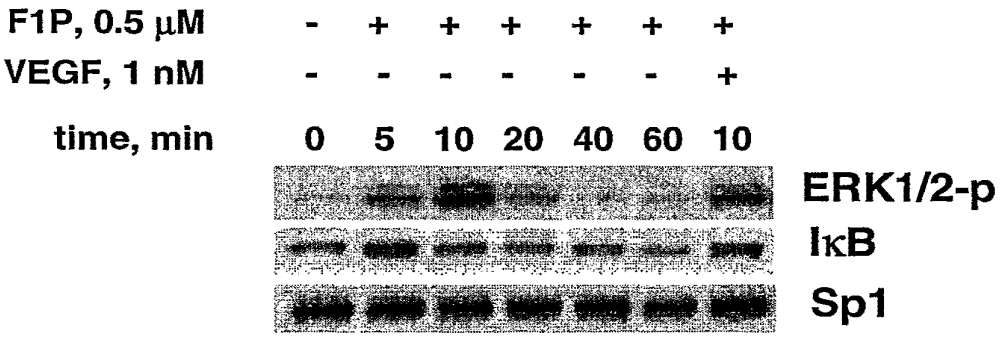

F1P, 0.5 µM - + + + + + +
VEGF, 1 nM - - - - - - +
time, min 0 5 10 20 40 60 10
ERK1/2-p
IκB
Sp1

Fig 6

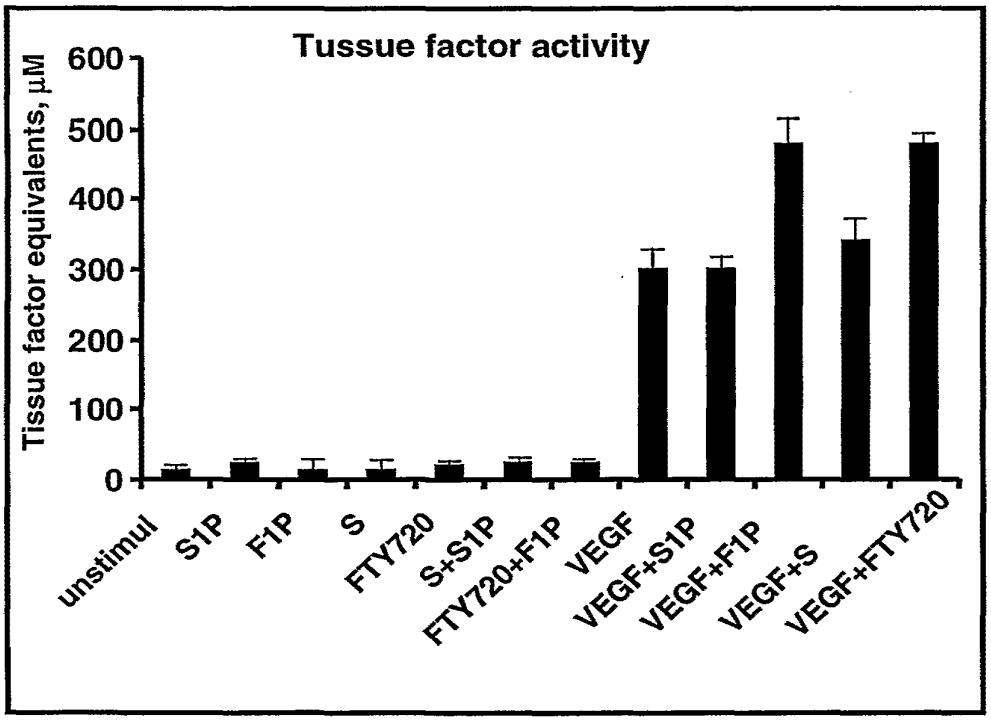
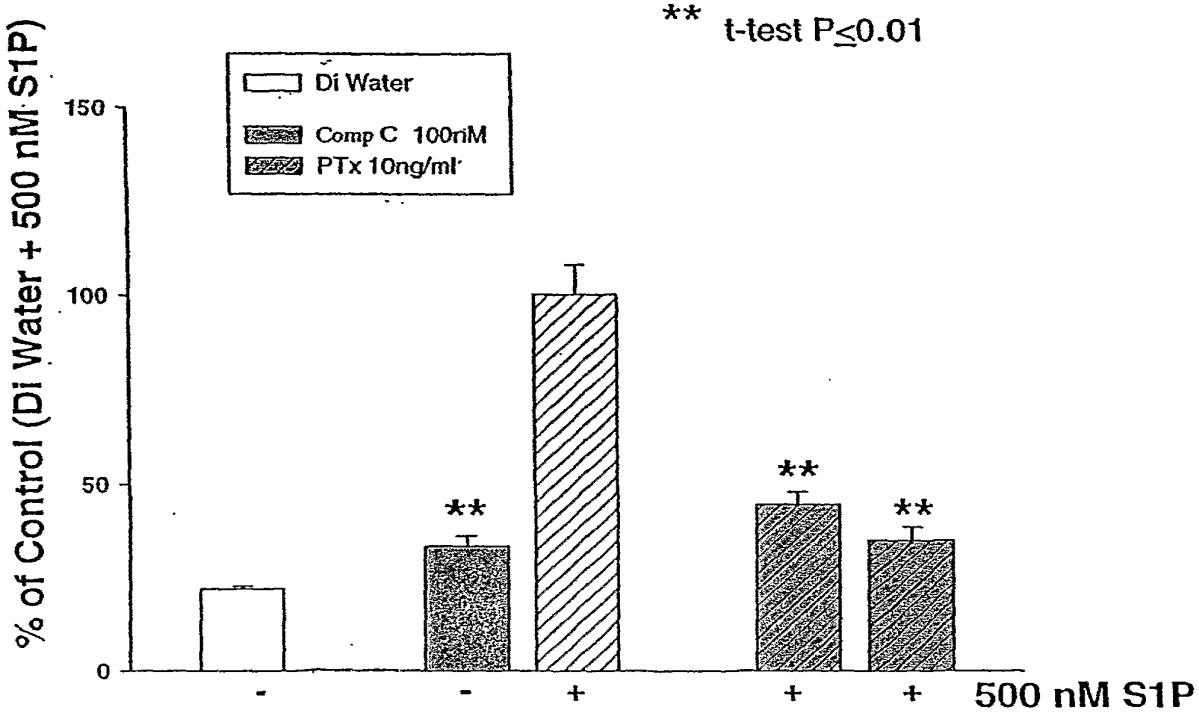

Tussue factor activity
Tissue factor equivalents, µM
600
500
400
300
200
100
0
unstimul
S1P
F1P
S
FTY720
S+S1P
FTY720+F1P
VEGF
VEGF+S1P
VEGF+F1P
VEGF+S
VEGF+FTY720

Fig 7


** t-test P≤0.01
Di Water
Comp C 100nM
PTx 10ng/ml
% of Control (Di Water + 500 nM S1P)
150
100
50
0
**
**
**
-
-
+
+
+
500 nM S1P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/05125

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/135 A61K31/381 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, CHEM ABS Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STEPKOWSKI, STANISLAW M.: "Molecular targets for existing and novel immunosuppressive drugs" INTERNET, 'Online! 21 June 2000 (2000-06-21), XP002254088 Retrieved from the Internet: <URL:http://www-ermm.cbcu.cam.ac.uk/000017 69a.pdf> 'retrieved on 2003-09-09! ---	
A	EP 1 195 165 A (ONO PHARMACEUTICAL CO) 10 April 2002 (2002-04-10) ---	

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

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
15 September 2003	26/09/2003

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

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

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	SOMPOL PERMPONGKOSOL ET ALL.: "Anticarcinogenic effect of FTY720 in human prostate carcinoma DU145 cells: modulation of mitogenic signaling, FAK, cell-cycle entry and apoptosis" INT.J.CANCER, vol. 98, 10 March 2002 (2002-03-10), pages 167-172, XP002254561 ---	
A	SUSAN PYNE ET ALL.: "SPHINGOSINE 1-PHOSPHATE SIGNALLING VIA THE ENDOTHELIAL DIFFERENTIATION GENE FAMILY OF G-PROTEIN-COUPLED RECEPTORS" PHARMACOLOGY & THERAPEUTICS, vol. 88, 2000, pages 115-131, XP002254562 ---	
A	HARUHITO AZUMA ET ALL.: "Marked Prevention of Tumor Growth and Metastasis by a Novel Immunosuppressive Agent, FTY720, in Mouse Breast" CANCER RESEARCH, vol. 62, 1 March 2002 (2002-03-01), pages 1410-1419, XP002254563 ------	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/05125

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1195165 A	10-04-2002	EP 1195165 A1	10-04-2002
		WO 0103739 A1	18-01-2001

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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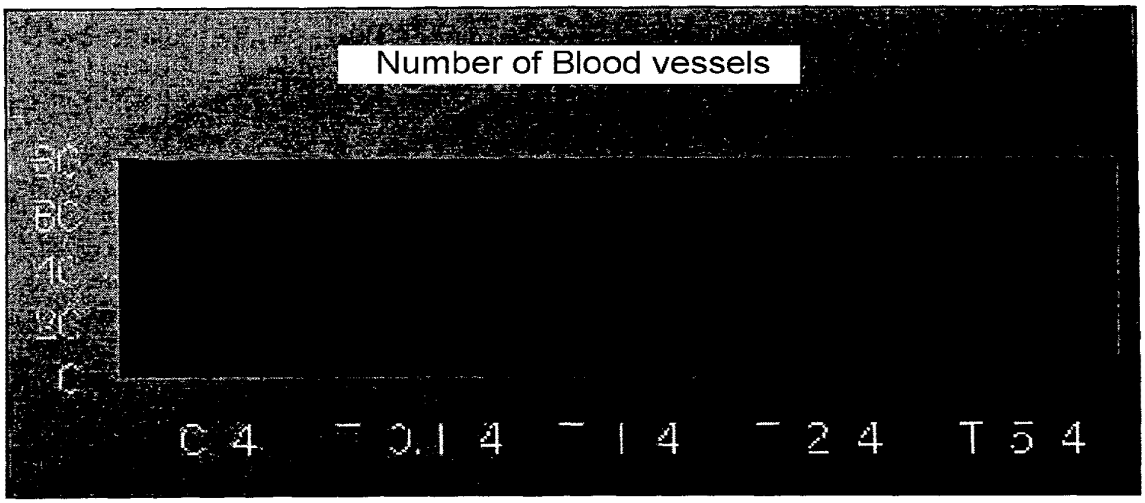
Published:
— *with international search report*

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

(54) Title: INHIBITOR OF ANGIOGENESIS AND KIT FOR TREATING CANCER COMPRISING THE INHIBITOR



(57) Abstract: The inhibitor of angiogenesis is characterized by containing tetraacetylphytosphingosine and the kit for inhibiting angiogenesis is characterized by the inhibitor. The inhibitor and kit are effective in treating and preventing disorders in connection with extremely increased angiogenesis such as angioma, tumor and psoriasis by effectively inhibiting angiogenesis. Furthermore, they are effective in inhibiting proliferation of cancer cells and metastasis of cancer without any adverse effect.

WO 2004/050073 A1

INHIBITOR OF ANGIOGENESIS AND KIT FOR TREATING CANCER COMPRISING THE INHIBITOR

TECHNICAL FIELD

The present invention relates to the inhibitor of angiogenesis comprising tetraacethylphytosphingosine derivatives and the kit for treating cancer comprising the inhibitor.

BACKGROUND ART

A process called angiogenesis which was started to be researched with observation of new formation of blood vessels in placenta in 1935 was found in various fields such as nidation, developing child in a mother's womb, wound healing, menstruation for women, arthritis, diabetic retinopathy or the like. As accumulating evidences have been found that there are many blood vessels and frequent bleeding around cancer cells and angiogenesis plays key roles in development of cancer and the growth and metastasis of cancer cells, various researches on the substances which inhibit angiogenesis are under progression. As a results of full-scale researches on angiogenesis which was carried out from the 1960s, the fact that the cause of the rapid proliferation of cancer cells is angiogenesis was found, and angiogenic factors started to be found in 1980s. A variety of angiogenesis inhibition factors and angiogenesis inhibitors were found in 1990s, and their potentiality as an inhibitor of cancer cell proliferation was expanded drastically. Nowadays, many anti-angiogenic strategies are being evaluated in clinical trials. These approaches offer new hope for the successful treatment of cancer.

For develpomant and proliferation of cancer cells, first growth phase related to tumor growth factors is observed. In this phase, various tumor growth factors and blood vessels formation factors are expressed significantly, and cancer cells and new blood vessels are formed. Following the growth phase, a phase that infiltration of cancer cells occurs starts. An disequilibrium between proteinases which digest

extracellular matrix and basement membrane, and inhibitory substances of proteinases occurs in this phase, wherein proteinases such as MMP-2 (matrixmetalloproteinase-2), MMP-9 (matrixmetalloproteinase-9), uPA (urokinase type plasminogen activator) or the like increas, whereas PAI-1 (plasminogen activator inhibitor-1), TIMP (tissue inhibitor of metalloproteinase) or the like involved in inhibition of proteinases decrease. Finally, in a phase that metastasis of cancer cells occurs, activity of cell adhesion molecule increases, thereby increasing adhesiveness of cells and metastasis of cancer cells occurs completely. Since such changed biological activities occurred in development and metastasis of cancer can be regulated by each of specific inhibitors, various studies on biological treatments based on above concepts are set about actively, and improvements of treatment effect through selective treatment are expected.

First, infiltration of cancer cells and angiogenesis require proteinases. Cancer cells, fibroblasts and endothelial cells produce proteinases and decompose extracellular matrix and basement membrane to give rise to infiltration of cancer cells and angiogenesis. Proteinases involved in such process include serine protease and MMPs(metrixmetalloproteinase). In process that such proteases decompose extracellular matrix, uPA(urokinase type plasminogen activator) converts plasminogen into plasmin to destroy fibrin, fibronectin, proteoglycan, laminin around cancer cells, and activates collagenase to decompose collagen. However, since uPA is inhibited by PAI-1(plasminogen activator inhibitor-1), PAI-1 is expected to control abilities of angiogenesis and metastasis of cancer cells.

In cancer cells, MMP-2 and MMP-9 are primarily activated. MMP-2 is activated by MMP presented in cell membrane of cancer cells, whereas MMP-9 is inhibited by TIMP. Therefore, recently it is expected that controlling the disequilibrium of MMP and TIMP is used effectively to inhibit formation of blood vessels, metastasis and infiltration, and thus it is suggested as a novel treatment concept.

When cancer cells are not supplied with nutrients through the formation of blood vessels, their growth is limited. Also, newly formed blood vessels are major

network for metastasis in addition to channel for suppliment of nutrients. The relationship between the degree of tumor angiogenesis and metastasis was well known, and it is established in various cancer cells that capillaries density of in situ tumor plays a key role in expectation of prognosis and metastasis of cancer. Recently, since monoclonal antibodies directed to angiogenic factors were developed, the degree of angiogenesis could be determined by measuring directly of angiogenic factors such as bFGF, VEGF, TGF-b or the like.

The concepts of cancer treatment through inhibition of angiogenesis are that correction of biological changes which are disturbed due to cancer as compared cancer cell with normal cell is to prevent differentiation, proliferation and metastasis of cancer cell, and as a result to block growth of cancer. Based on such concepts, MMP inhibitors have been synthesized and used for treating cancer since 1980s. However, according to the results of clinical tests, the effect of MMP inhibitors is far below what are expected. This is because the clinical tests are carried out on the subjects of which cancer has been already metastasized.

Angiogenesis is essential for proliferation and metastasis of tumor cells. Also, newly formed blood vessels are major network of metastasis of cancer cells. A proliferation and a migration of endothelial cells are events that occurred frequently in only cancer cells, except for wound healing for adults and menstruation for women. Consequently, angiogenesis that occurs in cancer tissues rather than normal tissues is a very selective target of cancer treatment, and in theory such treatment is considered to have little adverse effect and can be used together with other cancer treatments, thereby increasing effect of the treatment. As a result, substances to inhibit effectively angiogenesis can be used effectively to treat disorders in connection with extremely increased angiogenesis such as angioma, tumor and psoriasis.

DISCLOSURE OF THE INVENTION

An object of the present invention is treating and preventing disorders in connection with extremely increased angiogenesis such as angioma, tumor and psoriasis by effectively inhibiting angiogenesis. Furthermore, other object of the present invention is providing a pharmaceutical composition to inhibit proliferation of and metastasis of cancer cells effectively without any adverse effect.

To achieve above objects, the inhibitor of angiogenesis of the present invention is characterized by containing tetraacetylphytosphingosine.

Also, the kit for treating cancer of the present invention is characterized by comprising the inhibitor of angiogenesis containing tetraacetylphytosphingosinethe.

The kit for treating cancer is characterized by further comprising anti-cancer drug and irradiator.

For the kit for treating cancer, the anti-cancer drug is characterized by spingolipid derivatives.

For the kit for treating cancer, the spingolipid derivatives are one or more spingolipid derivatives selected from the group consisting of phytosphingosine, enacethylphytosphingosine, C6 phytoceramide, C8 phytosphingosine, dimethylsphingosine, dimethylphytosphingosine and sphingosine.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

FIG. 1 is a graph representing the number of blood vessels determined on 4 days after treatment of 0.1 μM, 1 μM, 2 μM and 5 μM of a solution containing tetraacetylphytosphingosine according to the present invention respectively in comparison with negative control.

FIG. 2 is a graph representing the area of granulation tissue determined on 4

days after treatment of 0.1 μM, 1 μM, 2 μM and 5 μM of a solution containing tetraacetylphytosphingosine according to the present invention respectively in comparison with negative control.

FIG. 3 is a graph representing a result of toxicity test of a solution containing tetraacetylphytosphingosine according to the present invention on HUVEC cell.

FIG. 4 is a graph representing a result of angiognesis test of a solution containing tetraacetylphytosphingosine according to the present invention.

FIG. 5 is photographes representing a result of angiognesis test of a solution containing tetraacetylphytosphingosine according to the present invention.

FIG. 6 is photographes representing that a solution containing tetraacetylphytosphingosine according to the present invention inhibited migration of endothelial cells.

FIG. 7 is a graph representing that a solution containing tetraacetylphytosphingosine according to the present invention inhibited migration of endothelial cells.

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention will now be described in more detail.

the inhibitor of angiogenesis and the kit for treating cancer comprising the same according to the present invention are described below in detail.

A composition according to the present invention comprises sphingolipid to inhibit angiogenesis very effectively. In particular, We found that acethylated derivatives of phytosphingosine, tetraacetylphytosphingosine inhibits angiogenesis and migration of HUVEC(Human Umbilical Vein Endothelial Cell) cell line strongly and it is effective in treating disorders in connection with extremely increased angiogenesis such as angioma, tumor and psoriasis by effectively inhibiting angiogenesis.

Angiogenesis in malignant tumor plays key roles that supply cancer cell with nutrients to allow cancer cell to grow rapidly, concurrently that function as migration

network for metastasizing cancer cell to another tissues and organs. Meanwhile, A number of newly formed blood vessels are observed in hyperkeratosis region of skin diseases such as psoriasis. In this respect, it is believed that an inhibitor of angiogenesis inhibits the growth of cancer cells and prevents the metastasis of cancer cells as well as has little adverse effect. Therefore, because it is expected that a inhibitor of angiogenesis increases the effect of cancer treatment significantly, many studies have been focused on a inhibitor of angiogenesis. Also, it is expected that when a inhibitor of angiogenesis is used in conjunction with various anticancer treatments in patients who have obvious goal of a treatment and is expected to take the effect of the treatment, the effect of treatment will be excellent.

Sphingolipid is well known as sunstances involved in signal transduction in cell and play an important role in proliferation, differentiation and apoptosis or programmed cell death of cell. Ceramide is a kind of sphingolipid, and is sphingosine-based signaling molecules that fatty acid is linked to sphingosine backbone. ceramide is known as 2nd messenger involved in stress signaling, senescence, cell death, etc. Ceramide is generated from hydrolysis of membrane sphingomyelin that enriched in the brain on receiving the signal from TNF-alpha, Fas, etc. and determine the destination of cell. Sphingomyelin is converted into ceramide by sphingomyelinase, and subsequently it is converted into sphingosine by removing the fatty acid from ceramide by ceramidase. Sphingosine is converted into sphingosine-1-phosphate by sphingosine kinase, and subsequently it is decomposed by lyase. It is known that ceramide and long chain base of sphingosine give rise to apoptosis, and sphingosine-1-phosphate that phosphate group is linked to sphingosine make a function to stimulate cell growth and cell proliferation. Since the balance of sphingosine and phosphated sphingosine within cell is involved in cell growth, cell proliferation and cell death, the change of sphingolipid concentration affects cell lethally. It is well known that most anticancer drugs have a mechanism that causes to apoptosis by increasing the amount of ceramide within cell. Also, It is known that since cancer cells have a different

metabolic pathway of ceramide as compared with normal cells, and thus the concentration of ceramide of normal cells is lower than that of cancer cell, apoptosis is not occur in cancer cells, and cancer cells can grow and proliferate rapidly. it has been found in various studied that ceramide and long chain bases of shhingosine cause cancer cells to apoptosis. Recently, It was found that various derivatives of phytosphingosine that is sphingolipid and is produced in yeast causes cancer cells to apoptosis.

Many studies have been performing which attempt to give rise to apoptosis of cancer cell by regulating the metabolism of ceramide. First, various chemotherapies and radition therapies which are carried out on cancer cells cause to formation of ceramide within cancer cells and as a result give rise to apoptosis of cancer cells. Also, other approach which gives rise to apoptosis of cancer cell is direct treatment of ceramide and long chain bases of sphingolipid to cancer cells. Further, still other approach which can be used effectively is to block the decomposition of ceramide by administering ceramidase inhibitor for preventing the decrease of ceramide contents, or to block conversion of sphingosine into sphingosine-1-phosphate by administering sphingosinekinase inhibitor such as DMS(dimethylsphingosine) for inhibiting the growth of cancer cells.

Sphingosine-1-phosphate is in large amount in blood and plays a role that transduces signals of various foreign responses. It is known that when blood vessels are injured due to wound, sphingosine-1-phosphate in blood that stimulates formation and migration of endothelial cells is secreted in large amount to heal wound rapidly. Also, it is a well known fact that SPC(sphingosinephosphorylcholine) which is shpingosine derivatives has a excellent effect on wound healing.

The effect of long chain bases of sphingosine to kill cancer cell, which were found in precedent various studies represents that sphingosine can be used effectively for treating cancer. However, substances that inhibit migration of endothelial cells and angiogenesis, and concurrently give rise to apoptosis are expected to have better effect

to inhibit growth of cencer cells and give rise to apoptosis.

Recently, it has been reported frequently that phytosphingosine produced in yeast like sphingosine give rise to apoptosis of cancer cell. It was found that derivatives of phytosphingosine(phytosphingosine, N-acethylsphingosine, tetraacethylsphingosine, C6 phytoceramide, C8 phytoceramide or the like) give rise to apoptosis in various cell lines including HaCat(keratinocyte), fibroblast, CHO(Chinese hamster ovarian cell), HL-60(Human leukemia), B16F10(Melanocyte cell line), U937(Monocyte) or the like and cancer cell lines(H460, A539 : lung cancer). Further, it is believed that phytosphingosine derivatives are involved in various inflammatory responses because of their inhibitory effect of protein kinase C and phospholipase D.

A compound of the present invention can be administered by any means that achieve its intended purpose. For example, administration can be by oral, parenteral, rectal, vaginal, topical, intravenous, intramuscular, intraperitoneal, subcutaneous or the like. The dosage administered of the active compound will be dependent upon the recipient, particular diseases or pathological states to be treated, the severity of diseases or pathological states, mode of administration and judgement of prescriber. A determination of the dosage based on the above factors is well known to those skilled in the art. Generally, the dosage is within range of about 0.01 mg/kg/day to about 2000 mg/kg/day, preferably 0.5 mg/kg/day to 2.5 mg/kg/day.

The compound of the present invention can be formulated into pharmaceutical compositions with suitable pharmaceutically acceptable carriers. The pharmaceutical compositions are manufactured in a manner that is, itself, known by using typical carriers(see, for example, E.W. Martin, Remington's Pharmaceutical Sciences, latest edition, Merck Publ. Co., Easton, PA). The compound of the present invention can be administered together with other anticancer drugs. Also, the pharmaceutical compositions can be administered in conjunction with other agents and treatments for treating diseases. For example, when the pharmaceutical compositions are administered, surgery, radiation therapy or chemotherapy can be also carried out

concurrently. According to the intended mode of administration, the pharmaceutical compositions can be in the forms of solid, semi-solid or liquid. The dosage forms include, but not limited to tablets, pills, capsules, suppositories, granules, small saccus, powders, creams, lotions, ointments, patches, liquid solutions, suspensions, dispersions, emulsions, syrups or the like. Also, the active ingredients can be encapsulated in liposome, microparticle or microcapsule.

General nontoxic carriers include, but are not limited to mannitol, lactose, starch, magnesium stearate, sodium saccharine, talc, cellulose, glucose, sucrose, dextrose, glycerol, magnesium carbonate, triglyceride, oil, solvent, sterile water, isotonic saline(pharmaceutical grade) and the like. Solid composition such as tablets, pills, granules or the like can be coated conveniently.

Typically, composition for administrating intraveneously is a solution in sterile isotonic buffer and contains topical anesthetic for alleviating pain at injection site. If desired, drug can contain small amount of nontoxic auxillaries such as wetting agent, emulsifier, pH buffer and the like. Examples of such auxillaries include, but are not limited to sodium acetate, sorbitan monolaurate, triethanolamine and triethanolamine oleate. Also, the composition according to the present invention comprises excipients such as stabilizer, antioxidant, binder, colorant, flavoring agent and thickening agent.

The inhibitor of angiogenesis according to the present invention comprises tetraacetylphytosphingosine, preferably 0.001 percent by weight to 99 percent by weight of tetraacetylphytosphingosine based on total composition. Less than 0.001 percent by weight of tetraacetylphytosphingosine has little effect, and 99 percent by weight or less represents that there is other additives or impurities in the composition.

The present invention will now be illustrated by the following preferred embodiments, but not limiting the scope of the invention.

Examples

We established that tetraacetylphytosphingosine caused cancer cell to apoptosis, and concurrently inhibited angiogenesis by determining apoptosis and inhibitory effect of angiogenesis of tetraacetylphytosphingosine as described below, and thus it can be used effectively for treating cancer.

<Example 1: In vivo wound healing assay>

Female New Zealand White rabbits(body weight 2.0 kg) were used as experimental animals in this assay. PBS solution containing 0.1% BSA(BSA-PBS solution) was prepared as control solution for negative control group(comparative example 1). sphingosylphosphorylcholine(comparative example 2), phytosphingosine(comparative example 3), N-acethylphytosphingosine(comparative example 4) as positive control group, and tetraacetyl phytosphingosine(example 1) were dissolved in ethanol or methanol respectively. Each portion of the solutions was added to silicone glass tube. Then, it was charged with N_2 gas, and added 0.1% BSA-PBS solution. After coupling them with water sonicator and vortex, these solutions were spotted on and injected intradermally to wound region of experimental animals. At the same time, to study an effect of the angiogenesis as concentrations of tetraacethylsphingosine, the animal was treated with 0.1 μM, 1 μM, 2 μM and 5 μM of tetraacethylsphingosine, and campared with the results. Experimental animals were put into special stainless cages which was designed suitably to assay experimental animals, anesthetized by injecting ketamine(3-4 mg/kg) intramuscularly. The hair and corneous tissue of innerside of both ears were removed with shaving and washing, and then were disinfected with 70% ethanol. Four wound regions per ear were formed by using 6 mm punch for skin histological examination(Stiefel, Germany) under sterile condition if possible, and each wound region was spotted or injected intradermally 30 - 50 $\mu\ell$ of the control solution or each treatment material. Wound regions were sealed with cathereep(Nichiban Co., Tokyo Japan) which was cut in size which are greater than that of wound region to prevent contamination of the wound regions and

formation of crust. Then, the wound regions were protected with 2 x 2 gauze, and ears of rabbit were bandaged with elastopore(Nichiban Co., Tokyo Japan). Subsequently, the rabbits were bred in a cage per a rabbit. After 48 hrs, same procedure was repeated. On 4 days and 8 days after forming wound regions, sacrificing the rabbits and treating tissue for histological study. For histological study, wound tissues were fixed with 10% formalin, cut it in half longitudinally and made paraffin block. Then, approximately 5 ㎛ of segment was made, attached it to slide and stained with hematoxylin and eosin to observe a change of epidermis and dermis, and stained with Massons Trichrome to observe a degree of collagen formation of granulation tissue.

First, stained tissue specimen was calibrated with ocular micrometer of optical microscope for image analysis. Then, After photographing each histological change aspects with digital camera under 40X and 100X objective lens and saving them on computer(Pentium III), image analysis was carried out with Scion Image for Windows software which was provided by (C) 2000 Scion corporation as follows: that is, degree of epidermis migration could be determined by measuring the distance from left to right boundary of wound, and thickness of newly formed epidermis could be determined by measuring the thickness of three spots in 1 mm of pitch and averaging measured values. To compare with degree of granulation tissue formation of dermis, three methods were used: 1) A method of measuring total area of newly formed granulation tissue and comparing with it; 2) A method of counting the number of cells such as fibroblast presented in six spots of center region of wound under high magnification (100X) and comparing with it; and 3) A method of determining degree of angiogenesis by counting the number of capillaries in granulation tissue in a same way as described above. If there was eschar, it wasn't determined. The data obtained from the negative control group, the positive control group and the experimental group were analyzed statistically by carrying out paired student's t test. The results were shown in FIG. 1, FIG. 2 and Table 1.

FIG. 1 is a graph representing the number of blood vessels determined on 4

days after treatment of 0.1 μM, 1 μM, 2 μM and 5 μM of a solution containing tetraacetylphytosphingosine according to the present invention respectively in comparison with negative control. In FIG. 1, C-4 represents a result of a negative control group which elapsed 4 days after treatment. T-0.1-4 represents a result of a experimental group which elapsed 4 days after treatment of 0.1 μM of tetraacethylphytosphingosine(TAPS). T-1-4, T-2-4 and T-5-4 represent results of experimental groups which elapsed 4 days after treatment of 1 μM, 2 μM and 5 μM of TAPS respectively. As shown in FIG. 1, tetraacethylphytosphingosine inhibited angiogenesis greatly.

FIG. 2 is a graph representing the area of granulation tissue determined on 4 days after treatment of 0.1 μM, 1 μM, 2 μM and 5 μM of a solution containing tetraacetylphytosphingosine according to the present invention respectively in comparison with negative control. In FIG. 2, C-4 represents a result of a negative control group which elapsed 4 days after treatment, and T-0.1-4, T-1-4, T-2-4 and T-5-4 represent results of experimental groups which elapsed 4 days after treatment of 0.1 μM, 1 μM, 2 μM and 5 μM of TAPS respectively. As shown in FIG. 2, a solution containing tetraacetylphytosphingosine according to the present invention decreased the area of granulation tissue.

Meanwhile, same experiments were carried out with 5 μM of the negative control group(comparative example 1), sphingosylphosphorylcholine (SPC)(comparative example 2), phytosphingosine (PS)(comparative example 3), N-acethylphytosphingosine (NAPS)(comparative example 4) and tetraacetylphytosphingosine (TAPS) as example(example 1) respectively, then the number of blood vessels and the area of granulation tissue were determined. The results were as follows:

Table 1

	Comparative example 1 (negative control)	Comparative example 2:SPC (5 μM)	Comparative example 3:PS (5 μM)	Comparative example 4:NAPS (5 μM)	Example 1:TAPS (5 μM)
Area of granul-ation tissue	100%	151%	112%	212%	76%
Number of blood vessels	100%	148%	106%	96%	31%

As shown in the Table 1, sphingosinephosphorylcholine (SPC) prompted increment of the number of blood vessels and the area of granulation tissue significantly, and tetraacetylphytosphingosine (SPC) inhibited increment of the number of blood vessels and the area of granulation tissue significantly.

<Exemple 2: Toxicity test and angiogenesis test on HUVEC>

HUVEC (human umbilical vein endothelial cell) which was used in this study was cultured as follows: Umbilical cord soaked in cold PBS were cut in 15-20 cm, and washing it thoroughly. Then, cannular was inserted into veins of umbilical cord at its both ends, and sutured umbilical cord and cannular tightly. 2-way stopcock was connected to each cannular which was tightly inserted to umbilical cord, and then 0.45 ㎛ milipore filter was connected to one stopcock. After washing veins with PBS, collagenase solution was added, and incubated for 6 mins at 37℃. After 6 mins, collagenase solution was removed and harvested from veins by introducing heparin solution(10 ml) into the veins, and harvested cells were centrifuged at 1500 rpm for 5 mins. Precipitated endothelial cells were suspended in 5 ml of M199 medium that does

not contain FBS. Above suspension procedure was repeated twice. Then, endothelial cells obtained in this manner were suspended in 5 ml of a medium which is suitable for growing endothelial cells, transferred to T25 flask which coated with gelatin, and incubated in 5% CO_2 incubator at 37℃.

Meanwhile, human fibroblasts which were used in this study were incubated as follows: Skin tissues were obtained by circumcision sterilely, and washed three times with Hanks balanced solution to remove epidermis and subcutaneous fat. Dermis tissues were cut in suitable size and placed them on the bottom of 35 mm culture dish. For depositing dermis tissues to the culture dish thoroughly, the culture dish was placed in incubator(5% CO_2, 37℃, Forma Scientific, Inc., Ohio, U.S.A.) for 5 mins, and then culture broth was added. After about 1-2 weeks, grown fibroblasts were treated with 0.25% trypsin solution and 0.02% EDTA solution for 3-5 mins, separated and passaged.

Toxicity of cell and growing capacity were determined as follows:

Various cell suspensions were stained with 0.5 % Tryphan Blue to count cell numbers. Corresponding medium was loaded to column 1 of 96 multi well plate(blank). To all columns excluding column 1 was added dividedly 180 ㎕ of cells conditioned in corresponding medium, and incubated in incubator at 37℃ for 12 - 24 hrs. 20 ㎕ of a medicament that is 10% of culture broth was added to experimental group, and PBS in equal amount was added to control group. After further incubating for 2 days, 40 ㎕ of MTT solution that the concentration had adjusted to 5 mg/ml with PBS(pH 7.4) was added, and further incubated in incubator at 37℃ for 4 hrs. And then the plate was centrifuged at 1500 rpm for 10 mins to discard the supernatant. 150 ㎕ of 100% DMSO was added to the plate with multi-channel pipette, and the plate was shaked in plate shaker. Finally, the absorbance was determined with ELISA reader at 540 nm. Angiogenesis test of HUVEC was carried out as follows:

Matrigel was prepared as follows: First, collagen solution was prepared by mixing commercially available acid-soluble porcine type I collagen(3.0 mg/ml), 5x

DMEM and buffer(0.05 N NaOH, 2.2% $NaHC0_3$,200 mM HEPES; 7:2:1), loaded to 24 well or 96 well(100 - 300 ㎕/well) dividedly, and then incubated for approximately 10 mins to form a gel due to polymerization of collagen. Primary culture was carried to obtain HUVEC(those which were passaged five times), removed cells at 37℃, washed cells with PBS, detached cells with trypsin/EDTA, loaded cells to wells(4 - 6 x 10^4 cells/well), and then further incubated for 12 - 18 hrs. The results were shown in FIG. 3 and FIG. 4.

FIG. 3 is a graph representing a result of toxicity test of a solution containing tetraacetylphytosphingosine according to the present invention on HUVEC cell. As shown in FIG. 3, apoptosis occurred at the concentration of 5 μM or more of TAPS dramatically.

FIG. 4 is a graph representing a result of angiognesis test of a solution containing tetraacetylphytosphingosine according to the present invention.

Also, FIGs. 5(a), 5(b) and 5(c) are photographs representing results of angiogenesis test of a solution containing tetraacetylphytosphingosine according to the present invention.

As shown in FIG. 4 and FIG. 5, for tube formation test, TAPS was inhibited effectively a formation of blood vessels at the concentration of 0.1 μM, and inhibited nearly completely a formation of blood vessels at the concentration of 1 μM.

<Example 3 : Migration assay of endothelial cells>

Transwell membranes were coated with 0.2% gelatin, and then were left for 12 hrs at 4℃. The membrane for control group was treated with BSA-PBS as described in the above examples, whereas the membranes for experimental group were treated with 0.1 μM, 1 μM and 5 μM of tetraacetylphytosphingosine solution according to the present invention respectively as described in the above examples. After incubating these membranes for 2 hrs, staining them with Diff Quick solution, and then observing them on slide glass. The results were shown in FIG. 6 and FIG. 7.

FIG. 6 is a graph representing that a solution containing tetraacetylphytosphingosine according to the present invention inhibited migration of endothelial cells.

FIG. 7 is photographs representing that a solution containing tetraacetylphytosphingosine according to the present invention inhibited migration of endothelial cells. As shown in FIG. 6 and FIG. 7, migration of endothelial cells were inhibited by TAPS, since the number of endothelial cells migrating to the opposite side of the membrane was reduced as increasing the concentration of TAPS.

The inhibitor of the present invention and the kit comprising it are effective in treating and preventing disorders in connection with extremely increased angiogenesis such as angioma, tumor and psoriasis by effectively inhibiting angiogenesis. Furthermore, they are effective in inhibiting proliferation of cancer cells and metastasis of cancer without any adverse effect.

Although preferred embodiments of the present invention have been described for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

WHAT IS CLAIMED IS:

1. An inhibitor of angiogenesis containing tetraacetylphytosphingosine.

2. A kit for treating cancer, comprising an inhibitor of angiogenesis containing tetraacetylphytosphingosinethe.

3. The kit for treating cancer according to claim 2, wherein further comprising anti-cancer drug and irradiator.

4. The kit for treating cancer according to claim 3, wherein the anti-cancer drug is spingolipid derivatives.

5. The kit for treating cancer according to claim 4, wherein the spingolipid derivatives are one or more spingolipid derivatives selected from the group consisting of phytosphingosine, enacethylphytosphingosine, C6 phytoceramide, C8 phytosphingosine, dimethylsphingosine, dimethylphytosphingosine and sphingosine.A composition for inhibiting the activity of tyrosinase which comprises N-acetylphytosphingosine as an active ingredient.

Fig. 1

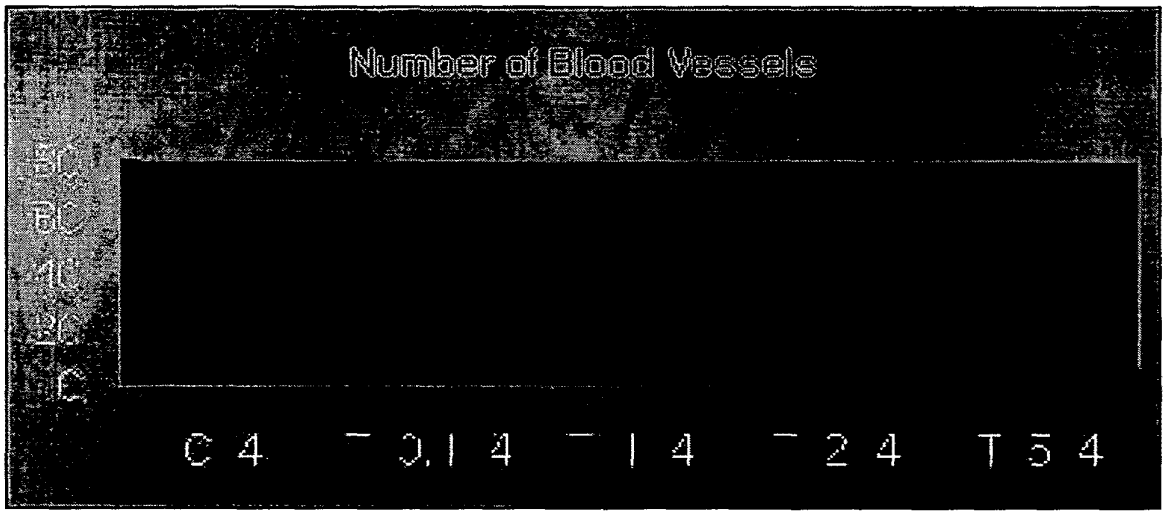

Number of Blood Vessels
C 4
T 5 4

Fig. 2

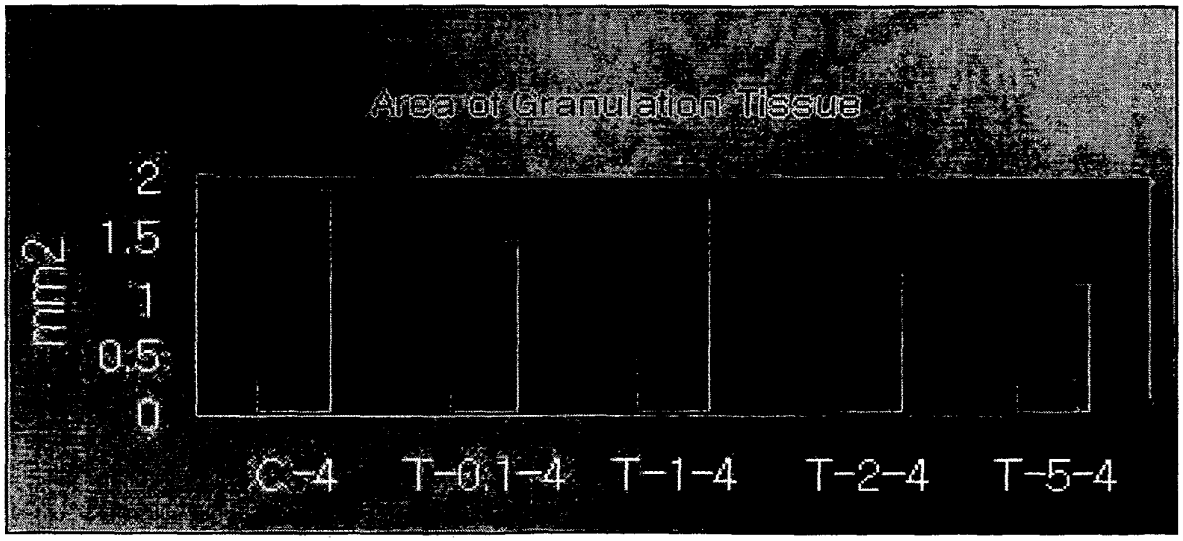

Area of Granulation Tissue
mm2
2
1.5
1
0.5
0
C-4
T-0.1-4
T-1-4
T-2-4
T-5-4

Fig. 3

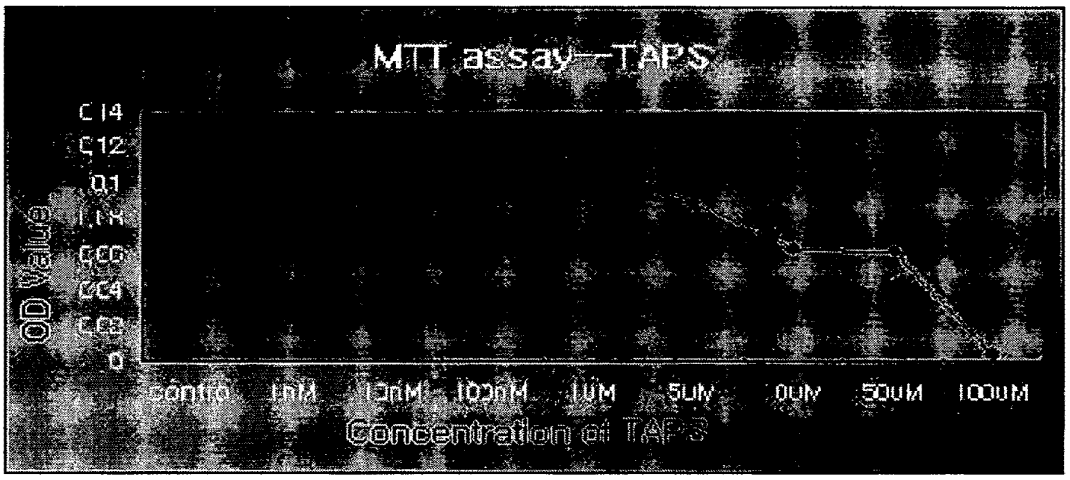

MTT assay--TAPS
OD Value
Concentration of TAPS

Fig. 4

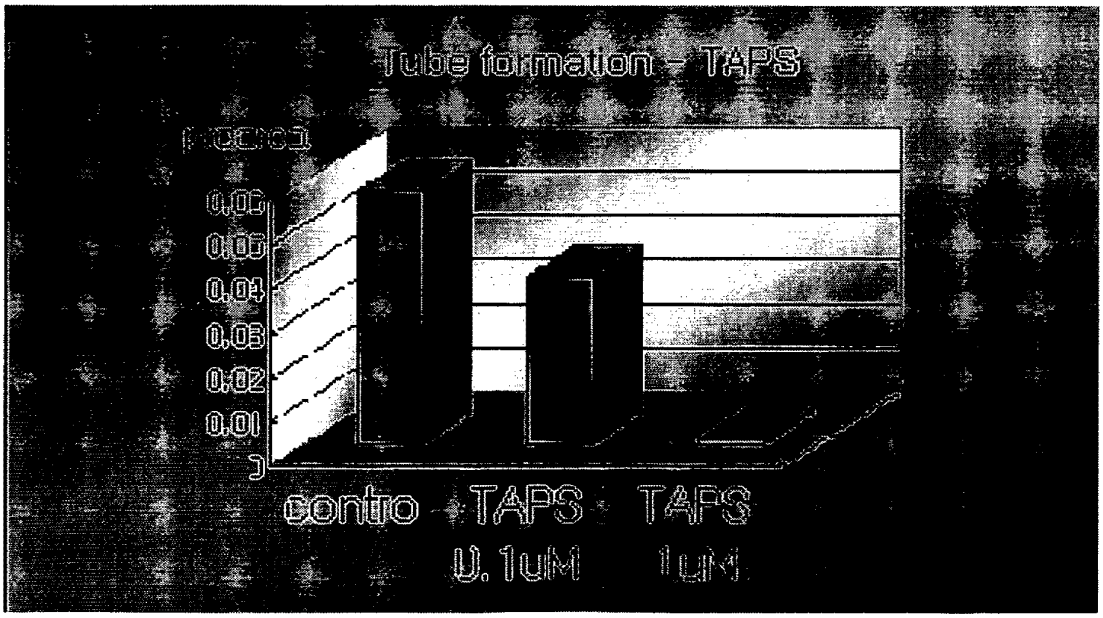

Tube formation - TAPS
0.05
0.04
0.03
0.02
0.01
TAPS
TAPS
0.1uM
1uM

Fig. 5

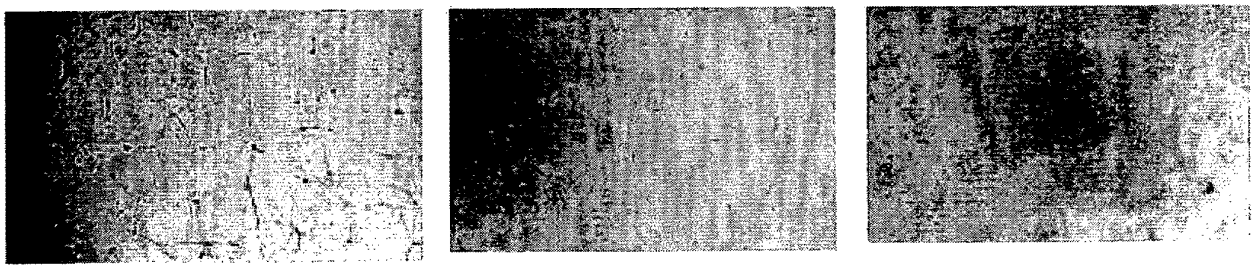

(a) Control
(b)TAPS 0.1uM
(c)TAPS 1uM

Fig. 6

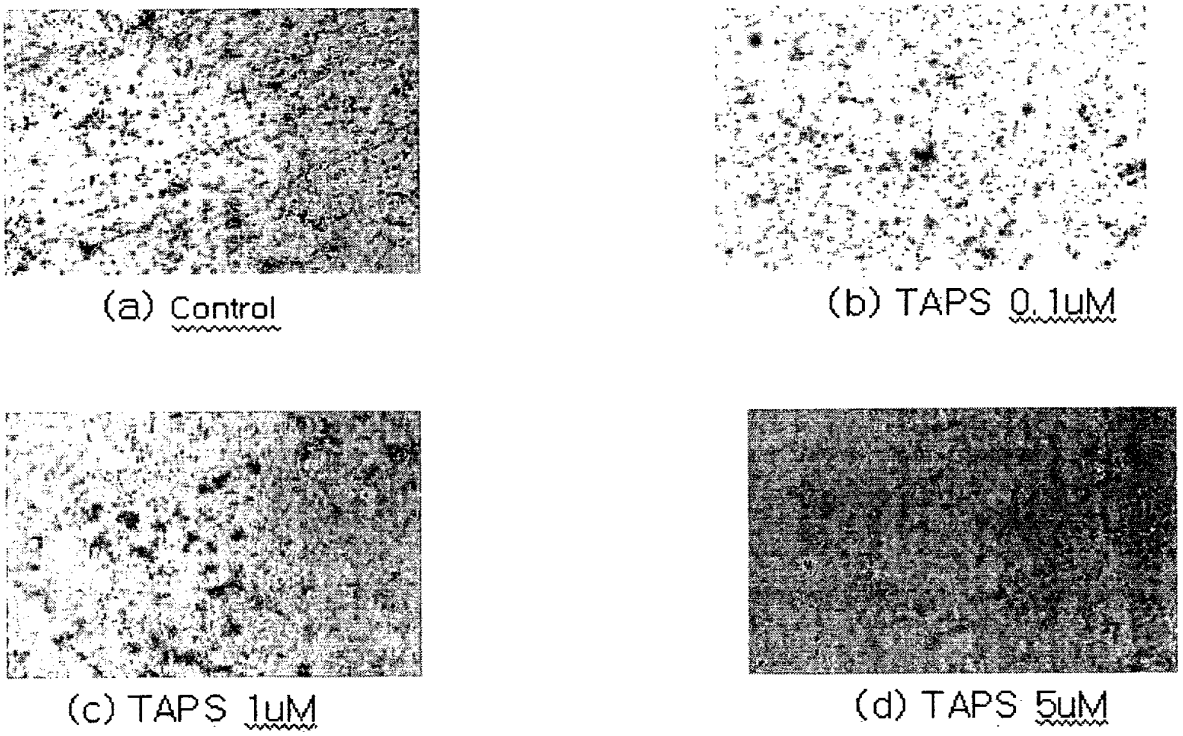
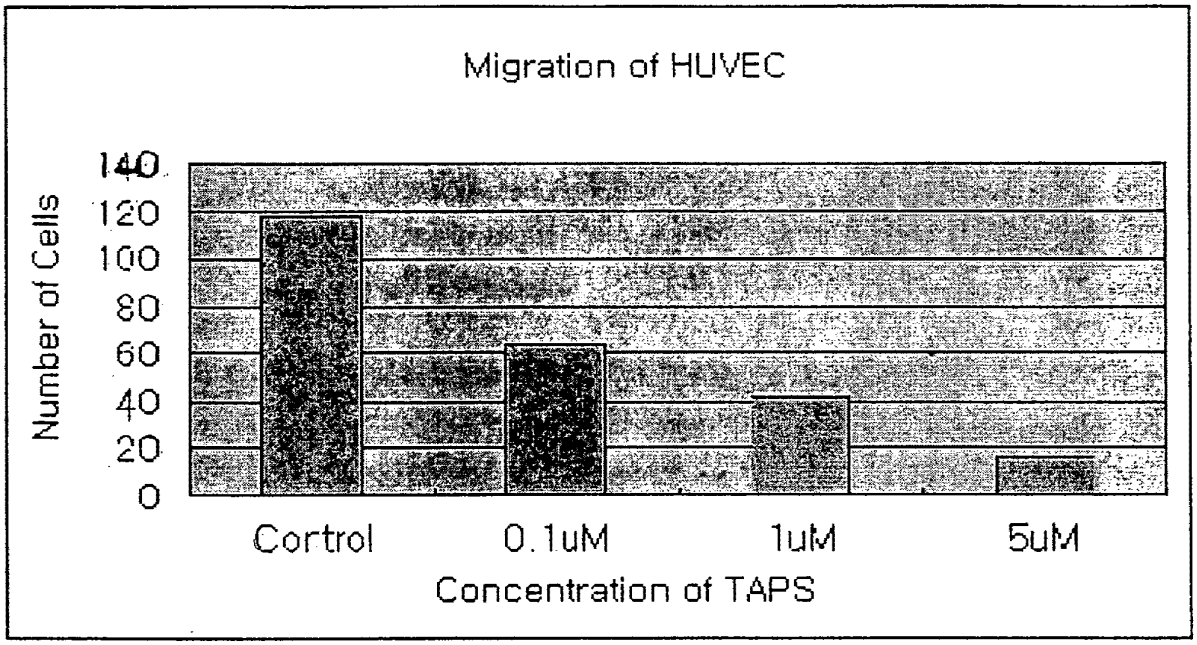

(a) Control
(b) TAPS 0.1uM
(c) TAPS 1uM
(d) TAPS 5uM

Fig. 7


Migration of HUVEC
Number of Cells
140
120
100
80
60
40
20
0
Cortrol
0.1uM
1uM
5uM
Concentration of TAPS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2003/002622

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 31/133

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 07 A61K 31/133

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patents and applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
STN(CA, Medline)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, A	WO 03/92667 A1(DOOSAN CORP.) 13 NOVEMBER 2003 See the whole document	1-5
A	XIAODONG SHU, et al., "Sphingosine kinase mediates Vascular Endothelial Growth Factor-Induced Activation of Ras and Mitogen-Activated Protein Kinases", Molecular and Cellular Biology, November 2002, 22(22), pp.7758-7768 See the whole document	1-5
A	CHAN WOO KIM, et al., "Extracellular Membrane Vesicles from Tumor Cells Promote Angiogenesis via Sphingomyelin", Cancer Research, November 2002, 62(21), pp.6312-6317 See the whole document	1-5
P, A	US 6610534 B2(NOVARTIS AG.) 26 AUGUST 2003 See the Abstract	1-5

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

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03/097631 A1	27.11.2003	KR 2042606 A	05.06.2002
US 6610534 B2	26.08.2003	WO 02/28406 A2	11.04.2002
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WO 2005/123104 A2

(54) Title: METHOD OF ADMINISTERING AND USING VEGF INHIBITORS FOR THE TREATMENT OF HUMAN CANCER

(57) Abstract: A method of treating a human patient suffering from cancer, comprising administering an effective amount of a vascular endothelial growth factor (VEGF) trap antagonist to the human patient, the method comprising: (a) administering to the patient an initial use of at least approximately 0.3 mglkg of the VEGF antagonist; and (b) administering the patient a plurality of subsequent doses of the VEGF antagonist in an amount that approximately the same or less of the initial dose, wherein the subsequent doses are separated in time from each other by at least one day. The methods of the invention are useful for treating a human cancer selected from the group consisting of renal cell carcinoma, pancreatic carcinoma, breast cancer, prostate cancer, colorectal cancer, malignant mesothelioma, multiple myeloma, ovarian cancer, and melanoma. The invention is further useful for treating a condition which benefits from the reduction of VEGFA and placental growth factor (PLGF).

METHOD OF ADMINISTERING AND USING VEGF INHIBITORS FOR THE TREATMENT OF HUMAN CANCER

Field of the Invention

[0001] The invention relates to methods of promoting regression of tumors and metastases by inhibiting vascular endothelial growth factor (VEGF) activity.

Description of Related Art

[0002] Vascular endothelial growth factor (VEGF) expression is nearly ubiquitous in human cancer, consistent with its role as a key mediator of tumor neoangiogenesis. Blockade of VEGF function, by binding to the molecule or its VEGFR-2 receptor, inhibits growth of implanted tumor cells in multiple different xenograft models (see, for example, Gerber et al. (2000) Cancer Res. 60:6253-6258). A soluble VEGF fusion protein antagonist, termed a "$VEGF_{R1R2}$ trap" or "VEGF trap" antagonist has been described (Kim et al. (2002) Proc. Natl. Acad. Sci. USA 99:11399-404; Holash et al. (2002) Proc. Natl. Acad. Sci. USA 99:11393-8.

Brief Summary of the Invention

[0003] In a first aspect, the invention features a method of treating a human patient suffering from cancer, comprising administering an effective amount of a vascular endothelial growth factor (VEGF) fusion protein trap antagonist to the human patient, the method comprising: (a) administering to the patient an initial dose of at least approximately 0.3 mg/kg of the VEGF trap antagonist; and (b) administering to the patient a plurality of subsequent doses of the VEGF trap antagonist in an amount that is approximately the same or less of the initial dose, wherein the subsequent doses are separated in time from each other by at least one day. The dosing regimen of the invention allows early attainment of an efficacious target trough serum concentration by providing an initial dose or doses of VEGF trap antagonist followed by subsequent doses of equal or smaller amounts of trap (greater front loading). The efficacious target trough serum concentration is reached in 4 weeks or less, preferably 3 weeks or less, more preferably 2 weeks or less, and most preferably 1 week or less, including 1 day or less. The target serum concentration is thereafter maintained by the administration of maintenance doses of equal or smaller amounts for the remainder of the treatment regimen or until suppression of disease symptoms is achieved.

[0004] In specific embodiments, the initial dose of the VEGF fusion protein antagonist is

in the range of approximately between 0.3 mg per kg body weight (mg/kg /kg) to 30 mg/kg. In a more specific embodiment, the initial dose is in the range of approximately 0.5 mg/kg to 10 mg/kg. In an even more specific embodiment, the initial dose is in the range of approximately 1 mg/kg to 6 mg/kg. Preferably, the cumulative weekly dose is in the range of 0.3 to 30 mg/kg.

[0005] In specific embodiments, at least one subsequent dose of the VEGF fusion protein antagonist is in the range of approximately between 0.3 mg/kg body weight to 30 mg/kg. In a more specific embodiment, at least one subsequent dose is in the range of approximately 0.5 mg/kg to 10 mg/kg. In an even more specific embodiment, at least one subsequent dose is in the range of approximately 1 mg/kg to 6 mg/kg.

[0006] In one embodiment, subsequent doses are separated in time from each other by at least 1 day, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, or at least 3 months. According to the invention, the cycle of dosing is preferably repeated as necessary to achieve suppression of the disease symptoms.

[0007] The method of the invention may be used to treat primary and/or metastatic tumors arising in the brain and meninges, oropharynx, lung and bronchial tree, gastrointestinal tract, male and female reproductive tract, muscle, bone, skin and appendages, connective tissue, spleen, immune system, blood forming cells and bone marrow, liver and urinary tract, and special sensory organs such as the eye. More specifically, the human patient treated by the method of the invention is a patient diagnosed with one of the following cancers: renal cell carcinoma, pancreatic carcinoma, breast cancer, prostate cancer, colorectal cancer, malignant mesothelioma, multiple myeloma, ovarian cancer, or melanoma. In a specific embodiment, the cancer being treated is renal cell carcinoma. In another embodiment, the cancer being treated is pancreatic carcinoma. In another embodiment, the cancer being treated is breast cancer. In another embodiment, the cancer being treated is colorectal cancer. In another embodiment, the cancer being treated is malignant mesothelioma. In another embodiment, the cancer being treated is multiple myeloma. In another embodiment, the cancer being treated is ovarian cancer. In another embodiment, the cancer being treated is melanoma. In another embodiment, the cancer being treated is non-small cell lung cancer. In another embodiment, the cancer being treated is prostate cancer.

[0008] The VEGF fusion protein trap antagonist is a dimer comprising two fusion proteins each composed of immunoglobulin (Ig)-like domains from two different VEGF receptors fused to a multimerizing component, wherein each fusion protein is capable of forming a higher order complex through interaction of multimerizing components on

different fusion proteins. The VEGF trap antagonist useful in the method of the present invention is a dimer capable of binding both vascular endothelial growth factor A (VEGFA) and placental growth factor (PLGF), and is selected from the group consisting of acetylated Flt-1(1-3)-Fc, Flt-1(1-$3_{R->N}$)-Fc, Flt-1(1-$3_{\Delta B}$)-Fc, Flt-1(2-$3_{\Delta B}$)-Fc, Flt-1(2-3)-Fc, Flt-1D2-VEGFR3D3-FcΔC1(a), Flt-1D2-Flk-1D3-FcΔC1(a), and VEGFR1R2-FcΔC1(a). In a specific and preferred embodiment, the VEGF trap antagonist is VEGFR1R2-FcΔC1(a) (also termed VEGF $trap_{R1R2}$) having the nucleotide sequence set forth in SEQ ID NO: 1 and the amino acid sequence set forth in SEQ ID NO: 2. The invention encompasses the use of a VEGF trap that is at least 90%, 95%, 98%, or at least 99% homologous with the nucleotide sequence set forth in SEQ ID NO: 1 and/or the amino acid sequence set forth in SEQ ID NO:2.

[0009] Administration of the agent may be by any method known in the art, including subcutaneous, intramuscular, intradermal, intraperitoneal, intravenous, intranasal, or oral routes of administration. In a preferred embodiment, the initial dose is administered by subcutaneous injection or intravenous injection. In further embodiments, the subsequent doses are administered by subcutaneous injection. In a preferred embodiment, the initial dose and at least one subsequent dose are administered by subcutaneous injection.

[0010] In a second aspect, the invention features a method of treating a human patient susceptible to or diagnosed with a disorder which is inhibited by an agent capable of blocking or inhibiting vascular endothelial growth factor A (VEGF A), wherein the agent capable of blocking or inhibiting VEGFA is a VEGF trap antagonist, the method comprising: (a) administering to the patient an initial dose of at least approximately 0.3 mg/kg of the VEGF trap; and (b) administering to the patient a plurality of subsequent doses of the VEGF trap in an amount that is approximately the same or less of the initial dose, wherein the subsequent doses are separated in time from each other by at least one day. In a specific and preferred embodiment, the VEGF trap antagonist is VEGFR1R2-FcΔC1(a) (also termed VEGF $trap_{R1R2}$) having the nucleotide sequence set forth in SEQ ID NO: 1 and the amino acid sequence set forth in SEQ ID NO: 2.

[0011] In a third embodiment, the invention features a therapeutic method of the invention optionally combined with a second chemotherapeutic agent. Chemotherapeutic agents combinable with administration of VEGF trap include, for example, anti-VEGF antibodies, anthracycline derivatives, such as doxorubicin or epirubicin taxol, and taxoid derivatives such as paclitaxel (Taxol®) and related derivatives.

[0012] Other objects and advantages will become apparent from a review of the ensuing detailed description.

Detailed Description

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

[0014] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0015] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

General Description

[0016] Vascular endothelial growth factor/vascular permeability factor (VEGF) was initially identified as a tumor-derived factor capable of increasing vascular permeability. It was subsequently found to be a proliferative factor for endothelial cells. In the embryo, VEGF is absolutely essential for the development of the vasculature. In the adult, VEGF is up-regulated in a variety of normal and pathological processes associated with increased vascular permeability and angiogenesis.

[0017] The family of VEGF-related angiogenic growth factors is comprised of VEGF itself (VEGF-A) and the related proteins VEGF-B, -C, -D and E, and placental growth factor (PLGF). In addition, there are at least four different isoforms of VEGF-A. However, as some members of the family have only recently been identified, their biological importance is still poorly understood. The actions of VEGF and its related factors are mediated by a group of three receptor tyrosine kinases, VEGFR1, VEGFR2 and VEGFR3.

[0018] The importance of VEGF in tumor angiogenesis has been demonstrated in a

number of animal models, where blocking VEGF signaling by a variety of strategies has proven effective at decreasing angiogenesis and inhibiting tumor growth (Gourley and Williamson (2000) Curr. Pharm. Des. 6:417-39). The permeability-inducing properties of VEGF are also of pathological importance, for example in edema formation, ascites and pleural effusions related to cancer. The degree of vascularization and of VEGF production have been proposed as prognostic factors for many types of solid and hematological malignancies (reviewed by Poon et al (2001) J. Clin. Oncol. 19:1207-1225).

[0019] Consistent with predictions from animal studies, blockade of VEGF using a humanized monoclonal antibody has emerged reporting promising results in cancer patients, based on preliminary reports from early clinical trials (Bergsland et al. (2000) ASCO Abstract #939). The VEGF fusion protein trap antagonist, because of its greater affinity for VEGF and its ability to bind other VEGF family members such as the PlGFs, is a potent and useful anti-cancer therapeutic agent.

Definitions

[0020] By the term "therapeutically effective dose" is meant a dose that produces the desired effect for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding). Efficacy can be measured in conventional ways, depending on the condition to be treated. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP), or determining the response rates (RR). Therapeutically effective amount also refers to a target serum concentration, such as a trough serum concentration, that has been shown to be effective in suppressing disease symptoms when maintained for a period of time.

[0021] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, prostate cancer, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

More Specifically, the methods of the invention are useful for treating any condition or disease which is ameliorated or inhibited with a VEGF inhibitor. Accordingly, when the disease or condition is cancer, the cancer treated by the method of the invention is one which is ameliorated or inhibited by administration of a VEGF inhibitor.

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

[0023] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

[0024] The term "serum concentration," "serum drug concentration," or "serum VEGF trap concentration" refers to the concentration of a drug, such as the VEGF fusion protein trap antagonist, in the blood serum or plasma of an animal or human patient being treated with the drug. Serum concentration is preferably determined by immunoassay. Preferably, the immunoassay is an ELISA according to the procedure disclosed herein.

[0025] The term "peak serum concentration" refers to the maximal serum drug concentration shortly after delivery of the drug into the animal or human patient, after the drug has been distributed throughout the blood system, but before significant tissue distribution, metabolism or excretion of drug by the body has occurred.

[0026] The term "trough serum concentration" refers to the serum drug concentration at a time after delivery of a previous dose and immediately prior to delivery of the next subsequent dose of drug in a series of doses. Generally, the trough serum concentration is a minimum sustained efficacious drug concentration in the series of drug administrations. Also, the trough serum concentration is frequently targeted as a minimum serum concentration for efficacy because it represents the serum concentration at which another dose of drug is to be administered as part of the treatment regimen. If the delivery of drug is by intravenous administration, the trough serum concentration is most preferably attained within 1 day of a front loading initial

drug delivery. If the delivery of drug is by subcutaneous administration, the peak serum concentration is preferably attained in 3 days or less. According to the invention, the trough serum concentration is preferably attained in 4 weeks or less, preferably 3 weeks or less, more preferably 2 weeks or less, most preferably in 1 week or less, including 1 day or less using any of the drug delivery methods disclosed herein.

[0027] The term "intravenous infusion" refers to introduction of a drug into the vein of an animal or human patient over a period of time greater than approximately 5 minutes, preferably between approximately 30 to 90 minutes, although, according to the invention, intravenous infusion is alternatively administered for 10 hours or less.

[0028] The term "subcutaneous administration" refers to introduction of a drug under the skin of an animal or human patient, preferable within a pocket between the skin and underlying tissue, by relatively slow, sustained delivery from a drug receptacle. The pocket may be created by pinching or drawing the skin up and away from underlying tissue.

[0029] The term "front loading" when referring to drug administration is meant to describe an initially higher dose followed by the same or lower doses at intervals. The initial higher dose or doses are meant to more rapidly increase the animal or human patient's serum drug concentration to an efficacious target serum concentration. According to the present invention, front loading is achieved by an initial dose or doses delivered over three weeks or less that causes the animal's or patient's serum concentration to reach a target serum trough concentration. Preferably, the initial front loading dose or series of doses is administered in two weeks or less, more preferably in 1 week or less, including 1 day or less. Most preferably, where the initial dose is a single dose and is not followed by a subsequent maintenance dose for at least 1 week, the initial dose is administered in 1 day or less. Where the initial dose is a series of doses, each dose is separated by at least 3 hours, but not more than 3 weeks or less, preferably 2 weeks or less, more preferably 1 week or less, most preferably 1 day or less.

The VEGF Fusion Protein Trap Antagonist

[0030] In a preferred embodiment, the VEGF trap is a receptor-Fc fusion protein consisting of the principal ligand-binding portions of the human VEGFR1 and VEGFR2 receptor extracellular domains fused to the Fc portion of human IgG1. Specifically, the VEGF trap antagonist consists essentially of Ig domain 2 from VEGFR1, which is fused to Ig domain 3 from VEGFR2, which in turn is fused to the Fc domain of IgG1 (SEQ ID

[0031] In a preferred embodiment, an expression plasmid encoding the VEGF trap is transfected into CHO cells, which secrete VEGF trap into the culture medium. The resulting VEGF trap is a dimeric glycoprotein with a protein molecular weight of 97 kDa and contains ~15% glycosylation to give a total molecular weight of 115 kDa.

[0032] Since the VEGF trap binds its ligands using the binding domains of high-affinity receptors, it has a greater affinity for VEGF than do monoclonal antibodies. The VEGF trap binds VEGF-A (K_D = 0.5 pM), PLGF1 (K_D = 1.3 nM), and PLGF2 (K_D = 50 pM); binding to other VEGF family members has not yet been fully characterized.

Treatment Population

[0033] The method of the invention may be used to treat tumors arising in the brain and meninges, oral pharynx, lung and bronchial tree, gastrointestinal tract, male and female reproductive tract, muscle, bone, skin, connective tissue, immune system, blood forming cells and bone marrow, liver and urinary tract, and special sensory organs such as the eye. More specifically, human patients suffering from renal cell carcinoma, pancreatic carcinoma, breast cancer, prostate cancer, colorectal cancer, malignant mesothelioma, multiple myeloma, ovarian cancer, or melanoma may be treated with the VEGF trap as described below.

Combination Therapies

[0034] In numerous embodiments, a VEGF fusion protein trap antagonist may be administered in combination with one or more additional compounds or therapies, including a second VEGF trap molecule. Combination therapy includes administration of a single pharmaceutical dosage formulation which contains a VEGF trap and one or more additional agents; as well as administration of a VEGF trap and one or more additional agent(s) in its own separate pharmaceutical dosage formulation. For example, a VEGF trap and a cytotoxic agent, a chemotherapeutic agent or a growth inhibitory agent can be administered to the patient together in a single dosage composition such as a combined formulation, or each agent can be administered in a separate dosage formulation. Where separate dosage formulations are used, the VEGF-specific fusion protein of the invention and one or more additional agents can be administered concurrently, or at separately staggered times, i.e., sequentially.

[0035] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to

include radioactive isotopes (e.g. I^{131}, I^{125}, Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

[0036] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (Cytoxan®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (Taxol®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (Taxotere®; Aventis

Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY 117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0037] A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially a cancer cell either *in vitro* or *in vivo*. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), Taxol ®, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C.

Pharmaceutical Compositions

[0038] Pharmaceutical compositions useful in the practice of the method of the invention include a therapeutically effective amount of an active agent, and a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium

chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[0039] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, or intramuscular administration to human beings. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0040] The active agents of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

Articles of Manufacture

[0041] In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container, a label and a package insert. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the

composition is a VEGF fusion protein trap antagonist. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes. In addition, the article of manufacture may comprise a package inserts with instructions for use, including, e.g., a warning that the composition is not to be used in combination with anthacycline-type chemotherapeutic agent, e.g. doxorubicin or epirubicin.

[0042] Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

Examples

[0043] The following example is put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Pharmacokinetics and Safety of the VEGF Fusion Protein Trap Antagonist in Primates

[0044] Preclinical toxicology studies were conducted with the VEGF trap (SEQ ID NO:2) in primates and rodents. Four- and thirteen week toxicology studies in cynomolgus monkeys showed that the VEGF trap was well tolerated when administered subcutaneously three times per week at doses of 1.5, 5, and 15 mg/kg (four week study), or twice a week at 1.5, 5, 15 or 30 mg/kg in the thirteen-week study. The VEGF trap was not highly immunogenic after four weeks in monkeys; only one mid-dose animal developed low titer antibodies.

Example 2: Treatment of Solid Tumors or Non-Hodgkin's Lymphoma.

[0045] Patients with refractory solid tumors or non-Hodgkin's lymphoma receiving no concurrent treatment for their cancer are treated with the VEGF trap as follows. The dose levels range from 0.3 mg/kg to 30 mg/kg given subcutaneously. Each patient receives a single initial dose of the VEGF trap followed by four weeks of observation and pharmacokinetic blood sampling. Beginning in the fifth week of the study, patients receive a series of 6 weekly injections at the assigned dose level. Plasma levels of the VEGF trap, and VEGF, both free and bound together as a complex, are monitored. Tumor burden is assessed at the beginning and end of the weekly dosing period and periodically during treatment; patients with stable disease, partial or complete responses may continue dosing for up to an additional 6 months in a continuation study. At higher dose levels where efficacy might be anticipated, patients undergo Dynamic Contrast Enhanced MRI scans to assess effects of VEGF trap administration on tumor perfusion.

We claim,

1. Use of a vascular endothelial growth factor (VEGF) antagonist comprising a dimer of two fusion polypeptides, each fusion polypeptide comprising (a) an immunoglobulin (Ig)-like domain 2 of a Flt-1 and Ig domain 3 of an Flk-1 or Flt-4, and (b) a multimerizing component, in the preparation of a medicament for treating a human patient suffering from cancer, by a method comprising:

(a) administering to the patient an initial dose of at least approximately 0.3 mg/kg of the VEGF antagonist; and

(b) administering to the patient a plurality of subsequent doses of the VEGF antagonist that are approximately the same or less than the initial dose, wherein the subsequent doses are separated in time from each other by at least one day.

2. Use according to claim 1, wherein the initial dose is from approximately 0.3 mg/kg to approximately 30 mg/kg; preferably from approximately 0.5 mg/kg to approximately 10 mg/kg.

3. Use according to claim 2, wherein the initial dose is approximately 1 mg/kg to approximately 6 mg/kg.

4. Use according to claim any one of the preceding claims, wherein the initial dose is approximately 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg and 6 mg/kg.

5. Use according to any one of the preceding claims, wherein the subsequent doses are the same or different and are from approximately 0.3 mg/kg to approximately 30 mg/kg; preferably from approximately 0.5 mg/kg to approximately 10 mg/kg.

6. Use according to claim 5, wherein the subsequent doses are from approximately 1 mg/kg, approximately 2 mg/kg, approximately 3 mg/kg, approximately 4 mg/kg, approximately 5 mg/kg and approximately 6 mg/kg.

7. Use according to any one of the preceding claims, wherein subsequent doses are separated in time from each other by at least 1 week.

8. Use according to claim 7, wherein subsequent doses are separated in time from each other by at least 1 month.

9. Use according to any one of the preceding claims, wherein the cancer is selected from renal cell carcinoma, pancreatic carcinoma, breast cancer, prostate cancer, colorectal cancer, malignant mesothelioma, multiple myeloma, ovarian cancer, and melanoma.

10. Use according to any one of the preceding claims, wherein the cancer is one that is inhibited by a vascular endothelial growth factor (VEGF) antagonist and a placental growth factor (PLGF) antagonist.

11. Use according to any one of the preceding claims, wherein the VEGF antagonist is selected from acetylated Flt-1(1-3)-Fc, Flt-1(1-$3_{R->N}$)-Fc, Flt-1(1-$3_{\Delta B}$)-Fc, Flt-1(2-$3_{\Delta B}$)-Fc, Flt-1(2-3)-Fc, Flt-1D2-VEGFR3D3-FcΔC1(a), Flt-1D2-Flk-1D3-FcΔC1(a), and VEGFR1R2-FcΔC1(a).

12. Use according to claim 11, wherein the VEGF antagonist is VEGFR1R2-FcΔC1 comprising the amino acid sequence of SEQ ID NO:2.

13. Use according to any one of the preceding claims, wherein the medicament is formulated for administration of the initial dose and subsequent doses by subcutaneous injection or intravenous injection.

14. A method of treating a human patient diagnosed with a cancer comprising administering an effective amount of a VEGF antagonist as defined in claim 1, 11 or 12, to the human patient, the method comprising:

(a) administering to the patient an initial dose of at least approximately 0.3 mg/kg of the VEGF antagonist; and

(b) administering to the patient a plurality of subsequent doses of the VEGF antagonist that are approximately the same as or less than the initial dose, wherein the subsequent doses are separated in time from each other by at least one week.

15. A method according to claim 14, wherein the initial dose and/or the subsequent doses are as defined in any one of claims 2 to 6.

16. A method according to claim 14 or 15, wherein the cancer is as defined in claim 9 or 10.

17. A method according to any one of claims 14 to 16, wherein subsequent doses are separated in time from each other by at least three weeks.

18. A method according to any one of claims 14 to 17 wherein the initial dose and/or the subsequent doses are administered by subcutaneous injection or intravenous injection.

SEQUENCE LISTING

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<120> Method of Administering and Using VEGF Inhibitors for the Treatment of Human Cancer

<130> 717A-WO

<140> To be Assigned
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(54) Title: ORGANIC COMPOUNDS

(57) Abstract: Disclosed are pharmaceutical combinations comprising at least one S1P receptor agonist, as well as a method for treating demyelinating diseases, e.g. multiple sclerosis or disorders associated therewith or Guillain-Barré syndrome, comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of a) an S1P receptor agonist, and b) at least one co-agent shown to have clinical activity against at least one symptom of a demyelinating disease.

Organic Compounds

The present invention relates to pharmaceutical combinations comprising at least one S1P receptor agonist and their uses in treating demyelinating diseases, e.g. multiple sclerosis and disorders associated therewith.

Multiple sclerosis is an immune-mediated disease of the central nervous system white matter with chronic inflammatory demyelination leading to progressive decline of motor and sensory functions and permanent disability. Manifestations of clinical disease usually begin in early adulthood, with women outnumbering men 2:1. The therapy of multiple sclerosis is only partially effective, and in most cases only offers a delay in disease progression despite anti-inflammatory and immunosuppressive treatment. Clinicians usually categorize patients into four types of disease patterns:

- *Relapsing-remitting* (RR-MS): Discrete motor, sensory, cerebellar or visual attacks that occur over 1-2 weeks and often resolve over 1-2 months, with or without treatment. Some patients accrue disability with each episode, yet remain clinically stable between relapses. About 85% of patients initially experience the RR form of MS, but within 10 years about half will develop the secondary progressive form.
- *Secondary-progressive* (SP-MS): Initially RR followed by gradually increasing disability, with or without relapses. Major irreversible disabilities appear most often during SP.
- *Primary-progressive* (PP-MS): Progression disease course from onset without any relapses or remissions, affecting about 15% of MS patients.
- *Progressive-relapsing* (PR-MS): Progressive disease from onset with clear acute relapses; periods between relapses characterized by continuing progression.

Accordingly, there is a need for agents which are effective in the treatment of demyelinating diseases, e.g. multiple sclerosis or Guillain-Barré syndrome, e.g. including reduction of, alleviation of, stabilization of or relief from the symptoms or illness which affect the organism.

It has now been found that a combination comprising at least one S1P receptor agonist and a co-agent, e.g. as defined below, has a beneficial effect on demyelinating diseases, e.g. multiple sclerosis and the disorders associated therewith.

In accordance with the particular findings of the present invention, there is provided

1. A pharmaceutical combination comprising:

a) an S1P receptor agonist, and

b) at least one co-agent shown to have clinical activity against at least one demyelinating disease symptom, e.g. a multiple sclerosis symptom or a symptom of Guillain-Barré syndrome.

2. 1 A method for treating a demyelinating disease, e.g. multiple sclerosis or disorders associated therewith or Guillain-Barré syndrome, comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of an S1P receptor agonist, e.g. a compound of formulae I to VII as defined hereinafter, and at least one co-agent, e.g. as indicated hereinafter.

2.2 A method for alleviating or delaying progression of the symptoms of a demyelinating disease, e.g. multiple sclerosis or Guillain-Barré syndrome, comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of an S1P receptor agonist, e.g. a compound of formulae I to VII as defined herein after, and at least one co-agent, e.g. as indicated hereinafter.

An early symptom of multiple sclerosis is optic neuritis. Accordingly, the present invention also provides

2.3 A method for treating, alleviating or delaying progression of optic neuritis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an S1P receptor agonist, e.g. a compound of formulae I to VII as specified herein after, e.g. Compound A or B or a pharmaceutically acceptable salt thereof.

3. A pharmaceutical combination as disclosed herein for use in any one of the methods 2.1 to 2.3.

4.1 A pharmaceutical composition for treating, alleviating or delaying progression of optic neuritis comprising an S1P receptor agonist, e.g. a compound of formulae I to VII as defined herein after, e.g. Compound A or B, together with one or more pharmaceutically acceptable diluents or carriers therefor.

4.2 A compound of formulae I to VII as defined herein after, e.g. Compound A or B, for use in the treatment, alleviating or delay of progression of optic neuritis.

4.3 An S1P receptor agonist, e.g. a compound of formulae I to VII as defined herein after, e.g. Compound A or B, for use in the preparation of a medicament for use in the treatment, alleviating or delay of progression of optic neuritis.

5.1 Use of an S1P receptor agonist, e.g. a compound of formulae I to VII as defined herein after, e.g. Compound A or B, for the preparation of a medicament for treating, alleviating or delaying the progression of optic neuritis.

5.2 Use of a) a sphingosine-1-phosphate (S1P) receptor agonist, and b) at least one co-agent shown to have clinical activity against at least one symptom of a demyelinating disease, for the preparation of a pharmaceutical combination for treating, alleviating or delaying progression of the symptoms of a demyelinating disease, e.g. for the preparation of a pharmaceutical combination for separate, simultaneous or sequential use in such a method.

5.3 A pharmaceutical composition as disclosed herein for separate, simultaneous or sequential use in medicine, e.g. in a method as disclosed at 2.1 to 2.3.

The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients.

The term "fixed combination" as that term is used herein means that the active ingredients, e.g. the S1P receptor agonist and a co-agent, are both administered to a patient simultaneously in the form of a single entity or dosage. As an example, a fixed combination would be one capsule containing two active ingredients.

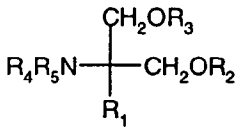
The term "non-fixed combination" as that term is used herein means that the active ingredients, e.g. the S1P receptor agonist and a co-agent, are both administered to a

patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the two compounds in the body, preferably at the same time. As an example, a non-fixed combination would be two capsules each containing one active ingredient where the purpose is to have the patient achieve treatment with both active ingredients together in the body.

An S1P receptor agonist is an immunomodulating compound which elicits a lymphopenia resulting from a re-distribution, preferably reversible, of lymphocytes from circulation to secondary lymphatic tissue, without evoking a generalized immunosuppression. Naïve cells are sequestered; CD4 and CD8 T-cells and B-cells from the blood are stimulated to migrate into lymph nodes (LN) and Peyer's patches (PP), and thus for example infiltration of cells into transplanted organs is inhibited.

Examples of appropriate S1P receptor agonists are, for example:

- Compounds as disclosed in EP627406A1, e.g. a compound of formula I



I

wherein R_1 is a straight- or branched (C_{12-22})carbon chain

- which may have in the chain a bond or a hetero atom selected from a double bond, a triple bond, O, S, NR_6, wherein R_6 is H, alkyl, aralkyl, acyl or alkoxycarbonyl, and carbonyl, and/or
- which may have as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxyimino, hydroxy or carboxy; or

R_1 is

- a phenylalkyl wherein alkyl is a straight- or branched (C_{6-20})carbon chain; or
- a phenylalkyl wherein alkyl is a straight- or branched (C_{1-30})carbon chain wherein said phenylalkyl is substituted by
- a straight- or branched (C_{6-20})carbon chain optionally substituted by halogen,
- a straight- or branched (C_{6-20})alkoxy chain optionally substitued by halogen,

- a straight- or branched (C_{6-20})alkenyloxy,
- phenylalkoxy, halophenylalkoxy, phenylalkoxyalkyl, phenoxyalkoxy or phenoxyalkyl,
- cycloalkylalkyl substituted by C_{6-20}alkyl,
- heteroarylalkyl substituted by C_{6-20}alkyl,
- heterocyclic C_{6-20}alkyl or
- heterocyclic alkyl substituted by C_{2-20}alkyl,

and wherein

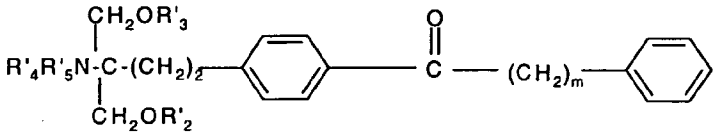
the alkyl moiety may have

- in the carbon chain, a bond or a heteroatom selected from a double bond, a triple bond, O, S, sulfinyl, sulfonyl, or NR_6, wherein R_6 is as defined above, and
- as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxy or carboxy, and

each of R_2, R_3, R_4 and R_5, independently, is H, C_{1-4} alkyl or acyl

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in EP 1002792A1, e.g. a compound of formula II

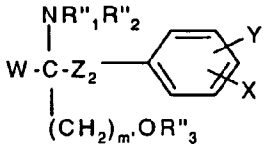


II

wherein m is 1 to 9 and each of R'_2, R'_3, R'_4 and R'_5, independently, is H, alkyl or acyl,

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in EP0778263 A1, e.g. a compound of formula III



III

wherein W is H; C_{1-6}alkyl, C_{2-6}alkenyl or C_{2-6}alkynyl; unsubstituted or by OH substituted phenyl; $R''_4O(CH_2)_n$; or C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH;

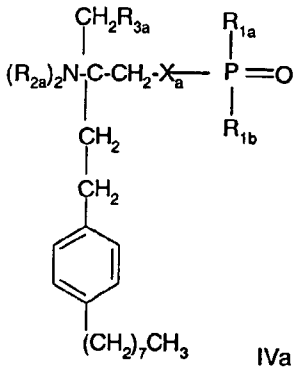
X is H or unsubstituted or substituted straight chain alkyl having a number p of carbon atoms or unsubstituted or substituted straight chain alkoxy having a number (p-1) of carbon atoms, e.g. substituted by 1 to 3 substitutents selected from the group consisting of C_{1-6} alkyl, OH, C_{1-6}alkoxy, acyloxy, amino, C_{1-6}alkylamino, acylamino, oxo, haloC_{1-6}alkyl, halogen, unsubstituted phenyl and phenyl substituted by 1 to 3 substituents selected from the group consisting of C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl and halogen; Y is H, C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl or halogen, Z_2 is a single bond or a straight chain alkylene having a number or carbon atoms of q,

each of p and q, independently, is an integer of 1 to 20, with the proviso of $6 \leq p+q \leq 23$, m' is 1, 2 or 3, n is 2 or 3,

each of R''_1, R''_2, R''_3 and R''_4, independently, is H, C_{1-4}alkyl or acyl,

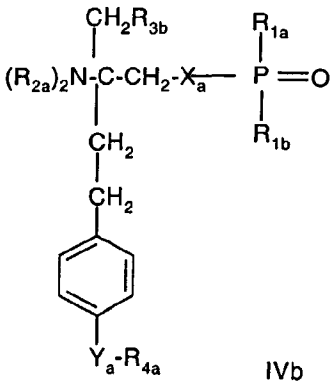
or a pharmaceutically acceptable salt thereof,

- Compounds as disclosed in WO02/18395, e.g. a compound of formula IVa or IVb



IVa

or



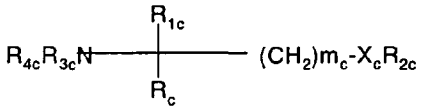
IVb

wherein X_a is O, S, NR_{1s} or a group $-(CH_2)_{na}-$, which group is unsubstituted or substituted by 1 to 4 halogen; n_a is 1 or 2, R_{1s} is H or (C_{1-4})alkyl, which alkyl is

unsubstituted or substituted by halogen; R_{1a} is H, OH, (C_{1-4})alkyl or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by 1 to 3 halogen; R_{1b} is H, OH or (C_{1-4})alkyl, wherein alkyl is unsubstituted or substituted by halogen; each R_{2a} is independently selected from H or (C_{1-4})alkyl, which alkyl is unsubstituted or substitued by halogen; R_{3a} is H, OH, halogen or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; and R_{3b} is H, OH, halogen, (C_{1-4})alkyl wherein alkyl is unsubstituted or substituted by hydroxy, or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; Y_a is $-CH_2-$, -C(O)-, -CH(OH)-, -C(=NOH)-, O or S, and R_{4a} is (C_{4-14})alkyl or (C_{4-14})alkenyl;

or a pharmaceutically acceptable salt or hydrate thereof;

- Compounds as disclosed in WO 02/076995, e.g. a compound of formula V



V

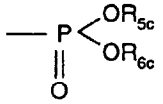
wherein

m_c is 1, 2 or 3;

X_c is O or a direct bond;

R_{1c} is H; C_{1-6} alkyl optionally substituted by OH, acyl, halogen, C_{3-10}cycloalkyl, phenyl or hydroxy-phenylene; C_{2-6}alkenyl; C_{2-6}alkynyl; or phenyl optionally substituted by OH;

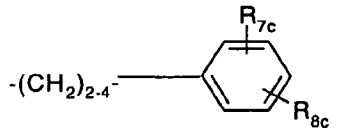
R_{2c} is



wherein R_{5c} is H or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms, and R_{6c} is H or C_{1-4}alkyl optionally substituted by halogen;

each of R_{3c} and R_{4c}, independently, is H, C_{1-4}alkyl optionally substituted by halogen, or acyl, and

R_c is C_{13-20}alkyl which may optionally have in the chain an oxygen atom and which may optionally be substituted by nitro, halogen, amino, hydroxy or carboxy; or a residue of formula (a)



(a)

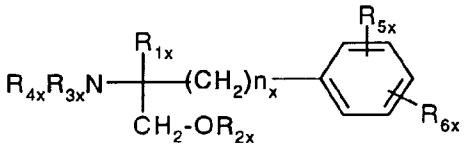
wherein R_{7c} is H, C_{1-4}alkyl or C_{1-4}alkoxy, and R_{8c} is substituted C_{1-20}alkanoyl, phenylC_{1-14}alkyl wherein the C_{1-14}alkyl is optionally substituted by halogen or OH, cycloalkylC_{1-14}alkoxy or phenylC_{1-14}alkoxy wherein the cycloalkyl or phenyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy, phenylC_{1-14}alkoxy-

C_{1-14}alkyl, phenoxyC_{1-14}alkoxy or phenoxyC_{1-14}alkyl,

R_c being also a residue of formula (a) wherein R_{8c} is C_{1-14}alkoxy when R_{1c} is C_{1-4}alkyl,

C_{2-6}alkenyl or C_{2-6}alkynyl,

or a compound of formula VI



VI

wherein

n_x is 2, 3 or 4

R_{1x} is H; C_{1-6}alkyl optionally substituted by OH, acyl, halogen, cycloalkyl, phenyl or hydroxy-phenylene; C_{2-6}alkenyl; C_{2-6}alkynyl; or phenyl optionally substituted by OH;

R_{2x} is H, C_{1-4} alkyl or acyl

each of R_{3x} and R_{4x}, independently is H, C_{1-4}alkyl optionally substituted by halogen or acyl,

R_{5x} is H, C_{1-4}alkyl or C_{1-4}alkoxy, and

R_{6x} is C_{1-20} alkanoyl substituted by cycloalkyl; cyloalkylC_{1-14}alkoxy wherein the cycloalkyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy;

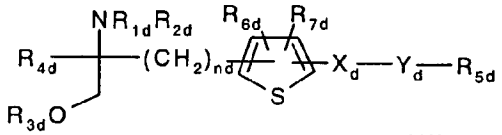
phenylC_{1-14}alkoxy wherein the phenyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy,

R_{6x} being also C_{4-14}alkoxy when R_{1x} is C_{2-4}alkyl substituted by OH, or pentyloxy or hexyloxy when R_{1x} is C_{1-4}akyl,

provided that R_{6x} is other than phenyl-butylenoxy when either R_{5x} is H or R_{1x} is methyl,

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in WO02/06268Al, e.g. a compound of formula VII



VII

wherein each of R_{1d} and R_{2d}, independently, is H or an amino-protecting group;

R_{3d} is hydrogen or a hydroxy-protecting group;

R_{4d} is lower alkyl;

n_d is an integer of 1 to 6;

X_d is ethylene, vinylene, ethynylene, a group having a formula – D-CH_2- (wherein D is carbonyl, – CH(OH)-, O, S or N), aryl or aryl substituted by up to three substitutents selected from group a as defined hereinafter;

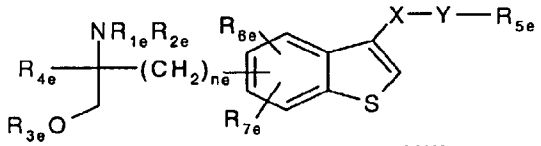
Y_d is single bond, C_{1-10}alkylene, C_{1-10}alkylene which is substituted by up to three substitutents selected from groups a and b, C_{1-10}alkylene having O or S in the middle or end of the carbon chain, or C_{1-10}alkylene having O or S in the middle or end of the carbon chain which is substituted by up to three substituents selected from groups a and b;

R_{5d} is hydrogen, cycloalkyl, aryl, heterocycle, cycloalkyl substituted by up to three substituents selected from groups a and b, aryl substituted by up to three substituents selected from groups a and b, or heterocycle substituted by up to three substituents selected from groups a and b; and

each of R_{6d} and R_{7d}, independently, is H or a substituent selected from group a;

<group a > is halogen, lower alkyl, halogeno lower alkyl, lower alkoxy, lower alkylthio, carboxyl, lower alkoxycarbonyl, hydroxy, lower aliphatic acyl, amino, mono-lower alkylamino, di-lower alkylamino, lower aliphatic acylamino, cyano or nitro;
<group b > is cycloalkyl, aryl, heterocycle, each being optionally substituted by up to three substituents selected from group a;
with the proviso that when R_{5d} is hydrogen, Y_d is a either a single bond or linear C_{1-10} alkylene, or a pharmacologically acceptable salt or ester thereof.

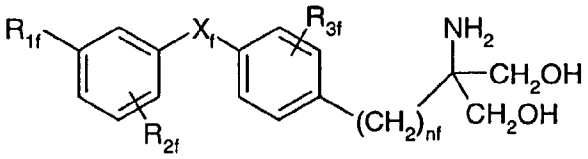
-Compounds as disclosed in JP-14316985 (JP2002316985), e.g. a compound of formula VIII:



VIII

wherein R_{1e},R_{2e},R_{3e},R_{4e},R_{5e},R_{6e},R_{7e}, n_e, X_e and Y_e are as disclosed in JP-14316985; or a pharmacologically acceptable salt or ester thereof.

-Compounds as disclosed in WO 03/29184 and WO 03/29205, e.g. compounds of formula IX



IX

wherein X_f is O or S, and R_{1f}, R_{2f}, R_{3f} and n_f are as disclosed in WO 03/29184 and O3/29205, e.g. 2-amino-2-[4-(3-benzyloxyphenoxy)-2-chlorophenyl]propyl-1,3-propane-diol or 2-amino-2-[4-(benzyloxyphenylthio)-2- chlorophenyl]propyl-1,3-propane-diol.

In each case where citations of patent applications are given, the subject matter relating to the compounds is hereby incorporated into the present application by reference.

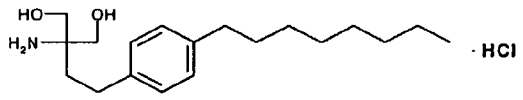
Acyl may be a residue R_y-CO- wherein R_y is C_{1-6}alkyl, C_{3-6}cycloalkyl, phenyl or phenyl-C_{1-4}alkyl. Unless otherwise stated, alkyl, alkoxy, alkenyl or alkynyl may be straight or branched.

When in the compounds of formula I the carbon chain as R_1 is substituted, it is preferably substituted by halogen, nitro, amino, hydroxy or carboxy. When the carbon chain is interrupted by an optionally substituted phenylene, the carbon chain is preferably unsubstituted. When the phenylene moiety is substituted, it is preferably substituted by halogen, nitro, amino, methoxy, hydroxy or carboxy.

Preferred compounds of formula I are those wherein R_1 is C_{13-20}alkyl, optionally substituted by nitro, halogen, amino, hydroxy or carboxy, and, more preferably those wherein R_1 is phenylalkyl substituted by C_{6-14}-alkyl chain optionally substituted by halogen and the alkyl moiety is a C_{1-6}alkyl optionally substituted by hydroxy. More preferably, R_1 is phenyl-C_{1-6}alkyl substituted on the phenyl by a straight or branched, preferably straight, C_{6-14}alkyl chain. The C_{6-14}alkyl chain may be in ortho, meta or para, preferably in para.

Preferably each of R_2 to R_5 is H.

A preferred compound of formula I is 2-amino-2-tetradecyl-1,3-propanediol. A particularly preferred S1P receptor agonist of formula I is FTY720, i.e. 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol in free form or in a pharmaceutically acceptable salt form (referred to hereinafter as Compound A), e.g. the hydrochloride, as shown:



A preferred compound of formula II is the one wherein each of R'_2 to R'_5 is H and m is 4, i.e. 2-amino-2-{2-[4-(1-oxo-5-phenylpentyl)phenyl]ethyl}propane-1,3-diol, in free form or in pharmaceutically acceptable salt form (referred to hereinafter as Compound B), e.g the hydrochloride.

A preferred compound of formula III is the one wherein W is CH_3, each of R''_1 to R''_3 is H, Z_2 is ethylene, X is heptyloxy and Y is H, i.e. 2-amino-4-(4-heptyloxyphenyl)-2-methyl-butanol, in free form or in pharmaceutically acceptable salt form (referred to

hereinafter as Compound C), e.g. the hydrochloride. The R-enantiomer is particularly preferred.

A preferred compound of formula IVa is the FTY720-phosphate (R_{2a} is H, R_{3a} is OH, X_a is O, R_{1a} and R_{1b} are OH). A preferred compound of formula IVb is the Compound C-phosphate (R_{2a} is H, R_{3b} is OH, X_a is O, R_{1a} and R_{1b} are OH, Y_a is O and R_{4a} is heptyl). A preferred compound of formula V is Compound B-phosphate.

A preferred compound of formula V is phosphoric acid mono-[(R)-2-amino-2-methyl-4-(4-pentyloxy-phenyl)-butyl]ester.

A preferred compound of formula VIII is (2R)-2-amino-4-[3-(4-cyclohexyloxybutyl)benzo[b]thien-6-yl]-2-methylbutan-1-ol.

When the compounds of formulae I to IX have one or more asymmetric centers in the molecule, the present invention is to be understood as embracing the various optical isomers, as well as racemates, diastereoisomers and mixtures thereof are embraced. Compounds of formula III or IVb, when the carbon atom bearing the amino group is asymmetric, have preferably the R-configuration at this carbon atom.

Examples of pharmaceutically acceptable salts of the compounds of the formulae I to IX include salts with inorganic acids, such as hydrochloride, hydrobromide and sulfate, salts with organic acids, such as acetate, fumarate, maleate, benzoate, citrate, malate, methanesulfonate and benzenesulfonate salts, or, when appropriate, salts with metals such as sodium, potassium, calcium and aluminium, salts with amines, such as triethylamine and salts with dibasic amino acids, such as lysine. The compounds and salts of the methods of the present invention encompass hydrate and solvate forms.

The co-agent b) may be selected from the following groups of compounds:

i) Interferons, e.g. pegylated or non-pegylated α-interferons, or β-interferons or τ–interferons, e.g. administered by subcutaneous, intramuscular or oral routes, preferably β-interferons;

ii) An altered peptide ligand such as Glatiramer, e.g. in the acetate form;

iii) Immunosuppressants with optionally antiproliferative/antineoplastic activity, e.g. mitoxantrone, methotrexate, azathioprine, cyclophosphamide, or steroids, e.g. methylprednisolone, prednisone or dexamethasone, or steroid-secreting agents, e.g. ACTH;

iv) Adenosine deaminase inhibitors, e.g. cladribine;

v) IV immunoglobulin G (e.g. as disclosed in Neurology, 1998, May 50(5):1273-81

vi) Monoclonal antibodies to various T-cell surface markers, e.g. natalizumab (ANTEGREN®) or alemtuzumab;

vii) TH2 promoting cytokines, e.g. IL-4, IL-10, or compounds which inhibit expression of TH1 promoting cytokines, e.g. phosphodiesterase inhibitors, e.g. pentoxifylline;

viii) Antispasticity agents including baclofen, diazepam, piracetam, dantrolene, lamotrigine, rifluzole, tizanidine, clonidine, beta blockers, cyproheptadine, orphenadrine or cannabinoids;

ix) AMPA glutamate receptor antagonists, e.g. 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline, [1,2,3,4,-tetrahydro-7-morpholin-yl-2,3-dioxo-6-(trifluoromethyl)quinoxalin-1-yl]methylphosphonate, 1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5H-2,3-benzodiazepine, or (-)1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-4,5-dihydro-3-methylcarbamoyl-2,3-benzodiazepine;

x) Inhibitors of VCAM-1 expression or antagonists of its ligand, e.g. antagonists of the $\alpha 4\beta 1$ integrin VLA-4 and/or alpha-4-beta-7 integrins, e.g. natalizumab (ANTEGREN®);

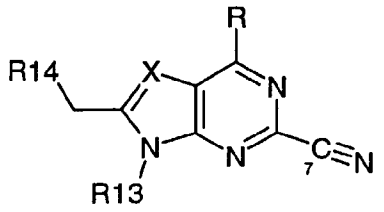
xi) Anti-Macrophage migration inhibitory factor (Anti-MIF);

xii) Cathepsin S inhibitors;

xiii) mTor inhibitors.

Cathepsin S inhibitors include e.g.:

a) a compound as disclosed in WO 03/20721, e.g. a compound of formula:



I

wherein

R is H, -R2, -OR2 or NR1R2,

wherein R1 is H, lower alkyl or C_3 to C_{10} cycloalkyl, and

R2 is lower alkyl or C_3 to C_{10} cycloalkyl, and

wherein each of R1 and R2 independently, is optionally substituted by halo, hydroxy, lower alkoxy, CN, NO_2, or optionally mono- or di-lower alkyl substituted amino;

X is =N- or =C(Z)-,

wherein Z is H, –C(O)-NR3R4, -NH-C(O)-R3, -CH_2-NH-C(O)-R3, -C(O)-R3, -S(O)-R3, -$S(O)_2$-R3,-CH_2-C(O)-R3, -CH_2-NR3R4, -R4, -C≡C-CH_2-R5, N-heterocyclyl, N-heterocyclyl-carbonyl, or –C(P)=C(Q)-R4

wherein

each of P and Q, independently, is H, lower alkyl or aryl,

R3 is aryl, aryl–lower alkyl, C_3-C_{10}cycloalkyl, C_3-C_{10}cycloalkyl-lower alkyl, heterocyclyl or heterocyclyl-lower alkyl,

R4 is H, aryl, aryl–lower alkyl, aryl-lower-alkenyl, C_3-C_{10}cycloalkyl, C_3-C_{10}cycloalkyl-lower alkyl, heterocyclyl or heterocyclyl-lower alkyl, or

wherein R3 and R4 together with the nitrogen atom to which they are joined to form an N-heterocyclyl group,

wherein N-heterocyclyl denotes a saturated, partially unsaturated or aromatic nitrogen containing heterocyclic moiety attached via a nitrogen atom thereof having from 3 to 8 ring atoms optionally containing a further 1, 2 or 3 heteroatoms selected from N, NR6, O, S, S(O) or $S(O)_2$ wherein R6 is H or optionally substituted (lower alkyl, carboxy, acyl (including both lower alkyl acyl, e.g. formyl, acetyl or propionyl, or aryl acyl, e.g. benzoyl), amido, aryl, S(O) or $S(O)_2$), and wherein the N-heterocyclyl is optionally fused in a bicyclic structure, e.g. with a benzene or pyridine ring, and wherein the N-heterocyclyl is optionally linked in a spiro structure with a 3 to 8

membered cycloalkyl or heterocyclic ring wherein the heterocyclic ring has from 3 to 10 ring members and contains from 1 to 3 heteroatoms selected from N, NR6, O, S, S(O) or $S(O)_2$ wherein R6 is as defined above), and

wherein heterocyclyl denotes a ring having from 3 to 10 ring members and containing from 1 to 3 heteroatoms selected from N, NR6, O, S, S(O) or $S(O)_2$ wherein R6 is as defined above), and

wherein each of R3 and R4, independently, is optionally substituted by one or more groups, e.g. 1-3 groups, selected from halo, hydroxy, oxo, lower alkoxy, CN or NO_2, or optionally substituted (optionally mono- or di-lower alkyl substituted amino, aryl, aryl-lower alkyl, N-heterocyclyl or N-heterocyclyl-lower alkyl (wherein the optional substitution comprises from 1 to 3 substituents selected from halo, hydroxy, lower alkoxy, CN, NO_2, or optionally mono- or di-lower alkyl substituted amino)), and

wherein

R5 is aryl, aryl-lower alkyl, aryloxy, aroyl or N-heterocyclyl as defined above, and

wherein R5 is optionally substituted by R7 which represents from 1 to 5 substitutents selected from halo, hydroxy, CN, NO_2 or oxo, or optionally substituted (lower-alkoxy, lower-alkyl, aryl, aryloxy, aroyl, lower-alkylsulphonyl, arylsulphonyl, optionally mono- or di-lower alkyl substituted amino, or N-heterocyclyl, or N-heterocyclyl-lower alkyl (wherein N-heterocyclyl is as defined above), and

wherein R7 is optionally substituted by from 1 to 3 substitutents selected from halo, hydroxy, optionally mono- or di- lower-alkyl substituted amino, lower-alkyl carbonyl, lower-alkoxy or lower-alkylamido;

R13 is lower alkyl, C3 to C10 cycloalkyl or C3-C10cycloalkyl-lower alkyl, all of which are independently optionally substituted by halo, hydroxy, CN, NO2 or optionally mono- or di-lower alkyl-substituted amino; and

R14 is H or optionally substituted (aryl, aryl-W-, aryl-lower alkyl-W-, C3 to C10 cycloalkyl, C3 to C10 cycloalkyl-W-, N-heterocyclyl or N-heterocyclyl-W- (wherein N-heterocyclyl is as defined above), phthalimide, hydantoin, oxazolidinone, or 2,6-dioxo-piperazine),

wherein –W- is –O-, -C(O)-, -NH(R6)-, -NH(R6)-C(O)-, -NH(R6)-C(O)-O-, (where R6 is as defined above),-S(O)-, $-S(O)_2-$ or –S-,

wherein R14 is optionally substituted by R18 which represents from 1 to 10 substitutents selected from halo, hydroxy, CN, NO_2, oxo, amido, carbonyl, sulphonamido, lower-alkyldioxymethylene, or optionally substituted (lower-alkoxy, lower-alkyl, lower-alkenyl, lower alkynyl, lower alkoxy carbonyl, optionally mono- or di-lower alkyl substituted amino, aryl, aryl-lower alkyl, aryl-lower alkenyl, aryloxy, aroyl, lower-alkylsulphonyl, arylsulphonyl, N-heterocyclyl, N-heterocyclyl-lower alkyl (wherein N-heterocyclyl is as defined above), heterocyclyl or R14 comprising aryl has aryl fused with a hetero-atom containing ring, and
wherein R18 is optionally substituted by R19 which represents from 1 to 4 substitutents selected from halo, hydroxy, CN, NO_2 or oxo, or optionally substituted (lower-alkoxy, lower-alkyl, lower-alkoxy-lower-alkyl, C_3-C_{10}cycloalkyl, lower-alkoxy carbonyl, halo-lower alkyl, optionally mono- or di-lower alkyl substituted amino, aryl, aryloxy, aroyl (e.g. benzoyl), acyl (e.g. lower-alkyl carbonyl), lower-alkylsulphonyl, arylsulphonyl or N-heterocyclyl, or N-heterocyclyl-lower alkyl (wherein N-heterocyclyl is as defined above)),

wherein R19 is optionally substituted by from 1 to 4 substitutents selected from halo, hydroxy, CN, NO_2, oxo, optionally mono- or di-lower alkyl substituted amino, lower-alkyl, or lower-alkoxy;

b) a compound as disclosed in WO 00/69855, e.g. N2-(3-furanylcarbonyl)-L-norleucine-2(S)-methyl-4-oxotetrahydrofuran-3(R)-yl amide;

c) a compound as disclosed in WO 01/19796, WO 01/19808, WO 02/51983, WO 03/24923, WO 03/24924, WO 03/41649 or WO 03/42197, e.g. N-(2-(1-cyanocyclopropylamino)-1(R)-(2-benzylsulfonylmethyl)-2-oxoethyl)morpholine-4-carboxamide, N-(2-(cyanomethylamino)-1-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)pyridine-4-carboxamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)-3,4-difluorobenzamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)-3-methylbenzamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)-1H-indole-5-carboxamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)-5-methylthiophene-2-carboxamide, N-(2-(4-cyano-1-methylpiperidin-4-ylamino)-1(R)-

(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)morpholine-4-carboxamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)-4-fluorobenzamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)thiophene-3-carboxamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)thiophene-2-carboxamide or N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)morpholine-4-carboxamide;

d) a compound as disclosed in WO 00/51998, WO 03/29200 or WO 03/37892, e.g. N-(1(S)-(N-(2-(benzyloxy)-1(R)-cyanoethyl)carbamoyl)-2-cyclohexylethyl)morpholine-4-carboxamide;

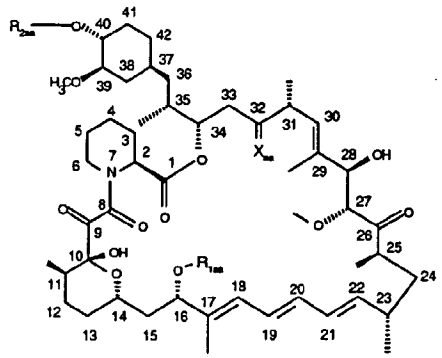
e) a compound as disclosed in WO 02/14314, WO 02/14315 or WO 02/14317, e.g. N1-(3-chloro-2-(4-(2-hydroxy-3-(5-(methylsulfonyl)-3-(4-(trifluoromethyl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo(4,3-pyridin-1-yl)propyl)piperazin-1-yl)phenyl)-N3-methylurea, 1-(1-(3-(3-(4-bromophenyl)-5-(methylsulfonyl)-4,5,6,7-tetrahydro-1H-pyrazolo(4,3-c)pyridine-1-yl)-2-hydroxypropyl)piperidin-4-yl)-6-chloro-1,2,3,4-tetrahydroquinolin-2-one, or 1-(5-(methylsulfonyl)-3-(4-(trifluoromethyl)phenyl-4,5,6,7-tetrahydro-1H-pyrazolo(4,3-c)pyridine-1-yl)-3-(4-(6-(4-morpholinyl)-1H-pyrrolo(3,2-c)pyridine-3-yl)piperidin-1-yl)propan-2-ol;

f) a compound as disclosed in WO 01/89451, e.g. 5-(2-morpholin-4ylethoxy)benzofuran-2-carboxylic acid ((S)-3-methyl-1-((S)-3-oxo-1-(2-(3-pyridin-2-ylphenyl)-acetyl)azepan-4-ylcarbamoyl)butylamide;

g) a compound as disclosed in WO 02/32879, WO 01/09169 or WO 00/59881A1, e.g. N-(1-benzothien-2-ylcarbonyl)-N-(2-(2-fluorophenyl)-4-oxo-1,2,3,4-tetrahydropyrimidin-5-yl)-L-leucinamide;

h) a compound as disclosed in WO 00/48992, WO 00/49007 or WO 00/49008.

The term "mTOR inhibitor" as used herein includes, but is not limited to rapamycin (sirolimus) or a derivative thereof. Rapamycin is a known macrolide antibiotic produced by Streptomyces hygroscopicus. Suitable derivatives of rapamycin include e.g. compounds of formula A



A

wherein

R_{1aa} is CH_3 or C_{3-6}alkynyl,

R_{2aa} is H or $-CH_2-CH_2-OH$, 3-hydroxy-2-(hydroxymethyl)-2-methyl-propanoyl or tetrazolyl, and

X_{aa} is =O, (H,H) or (H,OH)

provided that R_{2aa} is other than H when X_{aa} is =O and R_{1aa} is CH_3.

or a prodrug thereof when R_{2aa} is $-CH_2-CH_2-OH$, e.g. a physiologically hydrolysable ether thereof.

Compounds of formula A are disclosed e.g. in WO 94/09010, WO 95/16691, WO 96/41807, USP 5,362,718 or WO 99/15530 which are incorporated herein by reference. They may be prepared as diclosed or by analogy to the procedures described in these references.

Preferred rapamycin derivatives are 32-deoxorapamycin, 16-pent-2-ynyloxy-32-deoxorapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-rapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin and, more preferably, 40-0-(2-hydroxyethyl)-rapamycin. Further examples of rapamycin derivatives include e.g. CCI779 or 40- [3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate]-rapamycin or a pharmaceutically acceptable salt thereof, as disclosed in USP 5,362,718, ABT578 or 40-(tetrazolyl)-rapamycin, particularly 40-epi-(tetrazolyl)-rapamycin, e.g. as disclosed in WO 99/15530, or rapalogs as disclosed e.g. in WO 98/02441 and WO01/14387, e.g. AP23573 or TAFA-93.

In each case where citations of patent applications or scientific publications are given, the subject-matter relating to the compounds is hereby incorporated into the present application by reference. Comprised are likewise the pharmaceutically acceptable salts thereof, the corresponding racemates, diastereoisomers, enantiomers, tautomers as well as the corresponding crystal modifications of above disclosed compounds where present, e.g. solvates, hydrates and polymorphs, which are disclosed therein. The compounds used as active ingredients in the combinations of the invention can be prepared and administered as described in the cited documents, respectively. Also within the scope of this invention is the combination of more than two separate active ingredients as set forth above, i.e. a pharmaceutical combination within the scope of this invention could include three active ingredients or more. Further both the first agent and the co-agent are not the identical ingredient.

Utility of the compounds of formula I in treating demyelinating diseases, e.g. multiple sclerosis or Guillain-Barré syndrome as hereinabove specified, may be demonstrated in animal test methods as well as in clinic, for example in accordance with the methods hereinafter described. The most widely used animal model for multiple sclerosis is experimental autoimmune encephalomyelitis (EAE), based on shared histopathological and clinical features with the human disease.

A.1 In vivo: SJL/J Mouse model of chronic progressive EAE

The disease course in this animal model shares some common features with SP- and PR-MS. Immunization: On day 0 female SJL/J mice are immunized (subcutaneous flank injection) with 200 µl inoculum containing 500 µg bovine myelin basic protein (MBP) emulsified in complete Freund's adjuvant (CFA). On day 9 mice are boosted by a second MBP injection and an additional intravenous adjuvant injection consisting of 200 ng *B. pertussis* toxin. A final Pertussis injection is given on day 11.

Most of the MBP-immunized mice exhibit a severe bout of EAE by day 21. This is followed by a recovery phase starting around day 25, during which time mice remain symptom-free for about 20 days. Subsequently, by days 45-47, approximately 50% of the animals go into the progressive phase of the disease. Therefore, therapeutic

treatment with test compounds starts on day 21 when the disease is fully established and continues until day 70, unless stated otherwise. Recombinant mouse interferon β (INFβ Calbiochem/Biosciences) is dissolved in saline and given by intraperitoneal injection 3x per week. Compound (a), e.g. Compound A or B, is diluted in water and given p.o. 5x per week by gavage. Mice in the vehicle control group are MBP-immunized and treated with water.

Each experimental group consists of 10 mice, which are examined daily for clinical EAE symptoms. Disease incidence and the day of EAE onset also are recorded. Clinical grades of EAE are assessed using a scale from 0 to 3. Any disease-related mortality which occurs after starting drug treatment is recorded with a maximum score of 3.

Compound (a), e.g. Compound A or B at 0.6 mg/kg p.o. in combination with INFβ (10 000 IU) prevents disease progression for one month (days 45-75), compared to the vehicle-treated controls. In contrast, administration of INFβ alone (10 000 IU 3x/week) only marginally inhibits disease progression for about 1 week, after which the mice went on to develop a full EAE response that is indistinguishable from the disease course in vehicle-treated controls by day 68 onwards.

A.2 In vivo: Optic Neuritis in the DA rat model of chronic-protracted EAE

Ocular pathologic manifestations such as optic neuritis (neuromyelitis optica) are frequent in multiple sclerosis and often precede or accompany plaque formation in the brain white matter. Ocular areas, especially the optic chiasma, also are important targets in demyelinating forms of EAE. In such EAE models, functional disability caused by demyelination of the optic nerve can be assessed by electrophysiological methods, such as visual evoked cortical potentials and electroretinogram, in conjunction with morphological analysis of the ocular tissue.

Immunization: On day 0, female DA rats are immunized by a single intradermal injection at the tail base with 100-200 µl inoculum containing a recombinant encephalitogenic peptide, e.g. myelin oligodendrocyte glycoprotein, or a homogenate of syngeneic central nervous system tissue emulsified in one part CFA (volume:volume). Neurologic symptoms develop by 10 days post-immunization, and

clinical grades of EAE are assessed using a scale of 0 to 4. Therapeutic treatment with the test compound starts when the disease is fully established, usually on day 12, and continues for 2 weeks. Compound (a), e.g. Compound A or B at 0.3 mg/kg p.o. given once a day for 2 weeks, prevents disease progression for at least 2 months, compared to the vehicle-treated controls. Using combination treatment, suboptimal doses of Compound A or B (<0.1 mg/kg p.o.) and a mTOR inhibitor (<1 mg/kg p.o.) also curtail development of EAE symptoms and prevent disease-related weight loss after therapeutic dosing in the DA rat model. In a prophylactic treatment regimen, similar combinations of Compound A or B and a mTOR inhibitor prevent disease onset in the Lewis rat model of EAE, induced by an intradermal injection of guinea pig neuroantigen.

B Clinical Trial

Suitable clinical studies are, for example, open label, dose escalation studies in patients with multiple sclerosis. Such studies prove in particular the synergism of the active ingredients of the combination of the invention. The beneficial effects on multiple sclerosis can be determined directly through the results of these studies which are known as such to a person skilled in the art. Such studies are, in particular, suitable to compare the effects of a monotherapy using the active ingredients and a combination of the invention. Preferably, the dose of agent (a) is escalated until the Maximum Tolerated Dosage is reached, and the co-agent (b) is administered with a fixed dose. Alternatively, the agent (a) is administered in a fixed dose and the dose of co-agent (b) is escalated. Each patient receives doses of the agent (a) either daily or intermittent. The efficacy of the treatment can be determined in such studies, e.g., after 12, 18 or 24 weeks by evaluation of symptom scores every 6 weeks.

Alternatively, a placebo-controlled, double blind study can be used in order to prove the benefits of the combination of the invention mentioned herein.

The administration of a pharmaceutical combination of the invention results not only in a beneficial effect, e.g. a synergistic therapeutic effect, e.g. with regard to alleviating, delaying progression of or inhibiting the symptoms, but also in further surprising beneficial effects, e.g. fewer side-effects, an improved quality of life or a

decreased morbidity, compared with a monotherapy applying only one of the pharmaceutically active ingredients used in the combination of the invention.

A further benefit is that lower doses of the active ingredients of the combination of the invention can be used, for example, that the dosages need not only often be smaller but are also applied less frequently, which may diminish the incidence or severity of side-effects. This is in accordance with the desires and requirements of the patients to be treated.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

It is one objective of this invention to provide a pharmaceutical composition comprising a quantity, which is jointly therapeutically effective against multiple sclerosis or disorders associated therewith comprising a combination of the invention. In this composition, the first agent a) and co-agent (b) may be administered together, one after the other or separately in one combined unit dosage form or in two separate unit dosage forms. The unit dosage form may also be a fixed combination.

The pharmaceutical compositions for separate administration of the first agent a) and co-agent b) or for the administration in a fixed combination, i.e. a single galenical composition comprising at least two combination partners a) and b), according to the invention may be prepared in a manner known per se and are those suitable for enteral, such as oral or rectal, and parenteral administration to mammals (warm-blooded animals), including humans, comprising a therapeutically effective amount of at least one pharmacologically active combination partner alone, e.g. as indicated above, or in combination with one or more pharmaceutically acceptable carriers or diluents, especially suitable for enteral or parenteral application.

Suitable pharmaceutical compositions contain, for example, from about 0.1 % to about 99.9%, preferably from about 1 % to about 60 %, of the active ingredient(s).

Pharmaceutical preparations for the combination therapy for enteral or parenteral administration are, for example, those in unit dosage forms, such as sugar-coated tablets, tablets, capsules or suppositories, or ampoules. If not indicated otherwise, these are prepared in a manner known per se, for example by means of conventional mixing, granulating, sugar-coating, dissolving or lyophilizing processes. It will be appreciated that the unit content of a combination partner contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount can be reached by administration of a plurality of dosage units.

In particular, a therapeutically effective amount of each of the combination partner of the combination of the invention may be administered simultaneously or sequentially and in any order, and the components may be administered separately or as a fixed combination. For example, the method of delay of progression or treatment of multiple sclerosis or disorders associated therewith according to the invention may comprise (i) administration of the first agent a) in free or pharmaceutically acceptable salt form and (ii) administration of a co-agent b) in free or pharmaceutically acceptable salt form, simultaneously or sequentially in any order, in jointly therapeutically effective amounts, preferably in synergistically effective amounts, e.g. in daily or intermittently dosages corresponding to the amounts described herein. The individual combination partners of the combination of the invention may be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. Furthermore, the term administering also encompasses the use of a pro-drug of a combination partner that convert *in vivo* to the combination partner as such. The instant invention is therefore to be understood as embracing all such regimens of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

The effective dosage of each of the combination partners employed in the combination of the invention may vary depending on the particular compound or pharmaceutical composition employed, the mode of administration, the condition being treated, the severity of the condition being treated. Thus, the dosage regimen of the combination of the invention is selected in accordance with a variety of factors

including the route of administration and the renal and hepatic function of the patient. A physician, clinician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the single active ingredients required to alleviate, counter or arrest the progress of the condition. Optimal precision in achieving concentration of the active ingredients within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the active ingredients' availability to target sites, particularly when co-agent b) is a small molecule.

Daily dosages for the first agent a) will, of course, vary depending on a variety of factors, for example the compound chosen, the particular condition to be treated and the desired effect. In general, however, satisfactory results are achieved on administration of agent a) at daily dosage rates of the order of ca. 0.03 to 2.5 mg/kg per day, particularly 0.1 to 2.5 mg/kg per day, e.g. 0.5 to 2.5 mg/kg per day, as a single dose or in divided doses. The S1P receptor agonist, e.g. a compound of formulae I to VII, e.g. Compound A or B, may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration comprise from ca. 0.02 to 50 mg active ingredient, usually 0.1 to 30 mg, e.g. Compound A or B, together with one or more pharmaceutically acceptable diluents or carriers therefor. These dosages are also indicated when the S1P receptor agonist is used alone in the treatment of optic neuritis.

Interferons may be administered to a human in the following dosage ranges: interferon β–1b: up to 0.25 mg sc; interferon β–1a: up to 30 μg im; interferon α-2a: up to 10 million I.U. (MIU) sc or up to 1 MIU orally; interferon α-2b: up to 10 MIU sc or up to 1 MIU orally; pegylated interferon α-2a: up to 270 μg sc; pegylated interferon α-2b: up to 2.0 μg/kg sc.

Glatiramer may be administered to a human in a dosage range up to 20 mg sc, or up to 50 mg po.

Antineoplastic/antiproliferative immunosuppressants may be administered to a human in the forllowing dosage ranges: cyclophosphamide 500-1500 mg/m^2 IV; methotrexate up to 20 mg po; mitoxantrone 12 mg/m^2 IV, or azathioprine 2 mg/kg po.

Steroids may be administered to a human in the following dosage ranges: methylprednisolone 1-2-mg IV, or 24-48 mg po; prednisone 1 mg/kg po, or ACTH up to 100 MIU.

ADA inhibitors such as cladribine may be administered to a human in a dosage range up to 0.07 mg/kg/day.

IV Immunoglobulin G may be administered in a human in a dosage range up to 400 mg/kg IV.

Monoclonal antibodies to various T-cell surface markers may be administered in a human in the following dosage ranges: natalizumab up to 3mg/kg IV, alemtuzumab up to 30 mg sc or IV.

TH2 promoting cytokines may be administered to a human in the following dosage ranges: IL-4 up to 3μg/kg sc, or IL-10 up to 20μg/kg sc. Compounds which inhibit expression of TH1 promoting cytokines such as the phosphodiesterase inhibitor pentoxifylline may be administered in a human in a dosage range up to 4 mg po.

Antispasticity agents may be administered in a human in the following doage ranges: baclofen up to 100 mg po, diazepam up to 20 mg po, piracetam up to 24 mg po, dantrolene up to 100 mg po, lamotrigine up to 100 mg/day, riluzole up to 100 mg po, tizanidine up to 12 mg po, clonidine up to 0.1 mg po, β blockers (e.g. propanolol) up to 160 mg po, cyproheptadine up to 8 mg po, orphenadrine up to 100 mg po and cannabinoids (e.g. dronabinol) up to 5 mg po.

Cathepsin S inhibitors, e.g. a compound as disclosed in WO 03/20721, may be administered to a human in the dosage range 0.1 to 100 mg/kg/day.

mTor inhibitors, e.g. rapamycin or a derivative thereof, e.g. 40-O-(2-hydroxyethyl)-rapamycin, may be administered in a dosage range varying from about 0.1 to 25 mg/kg/day.

When used in treating, alleviating or delaying progression of optic neuritis, the S1P receptor agonist, e.g. a compound of formula I to VII, e.g. a compound A or B, may be administered systematically or topically, by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, topically, e.g. in the form of a topical ophthalmic composition, e.g. comprising an ophthalmic carrier. Pharmaceutical compositions comprising an S1P receptor agonist in association with at least one pharmaceutically acceptable carrier or diluent may be manufactured in conventional manner, e.g. by mixing the ingredients.

Compounds of formulae I to VII are well tolerated at dosages required for use in accordance with the present invention. For example, the acute LD_{50} is >10 mg/kg p.o. in rats and monkeys for Compound A.

CLAIMS

1. A pharmaceutical combination comprising:

a) a sphingosine-1-phosphate (S1P) receptor agonist, and

b) at least one co-agent shown to have clinical activity against at least one symptom of a demyelinating disease.

2. A pharmaceutical composition for treating, alleviating or delaying progression of optic neuritis comprising an S1P receptor agonist together with one or more pharmaceutically acceptable diluents or carriers therefor.

3. A combination or composition according to claim 1 or claim 2 wherein the S1P receptor agonist is selected from the compounds of formulae I to III, IVa, IVb, and V to VII substantially as described and defined herein.

4. A combination according to claim 1 or claim 3, wherein the co-agent b) is selected from the group consisting of interferons, altered peptide ligands, immunosuppressants, adenosine deaminase inhibitors, IV immunoglobulin G, monoclonal antibodies to T-cell surface markers, TH2 promoting cytokines, compounds which inhibit expression of TH1 promoting cytokines, antispasticity agents, AMPA glutamate receptor antagonists, inhibitors of VCAM-1 expression or antagonists of its ligand, anti-macrophage migration inhibitory factor, cathepsin S inhibitors and mTOR inhibitors.

5. A combination or composition according to any preceding claim, wherein the S1P receptor agonist is selected from 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, 2-amino-2-{2-[4-(1-oxo-5-phenylpentyl)phenyl]ethyl}propane-1,3-diol and their respective phosphate, in free form or in a pharmaceutically acceptable salt form.

6. A method for treating, alleviating or delaying progression of the symptoms of a demyelinating disease comprising co-administration of a therapeutically effective amount of a) an S1P receptor agonist, and b) at least one co-agent shown to have clinical activity against at least one symptom of a demyelinating disease.

7. A method for treating, alleviating or delaying progression of optic neuritis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an S1P receptor agonist.

8. A method according to claim 6 or 7 wherein the S1P receptor agonist is selected from a compound of formulae I to VII substantially as described and defined herein.

9. A method according to claim 6, 7 or 8 wherein the S1P receptor agonist is selected from 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, 2-amino-2-{2-[4-(1-oxo-5-phenylpentyl)phenyl]ethyl}propane-1,3-diol and their respective phosphate, in free form or in a pharmaceutically acceptable salt form.

10. A method according to claim 6, wherein the co-agent b) is selected from the group consisting of interferons, altered peptide ligands, immunosuppressants, adenosine deaminase inhibitors, IV immunoglobulin G, monoclonal antibodies to T-cell surface markers, TH2 promoting cytokines, compounds which inhibit expression of TH1 promoting cytokines, antispasticity agents, AMPA glutamate receptor antagonists, inhibitors of VCAM-1 expression or antagonists of its ligand, anti-macrophage migration inhibitory factor, cathepsin S inhibitors and mTOR inhibitors.

11. A combination or composition according to any of claims 1 to 5, for treating, alleviating or delaying progression of the symptoms of a demyelinating disease.

12. Use of a) a sphingosine-1-phosphate (S1P) receptor agonist, and b) at least one co-agent shown to have clinical activity against at least one symptom of a demyelinating disease, for the preparation of a pharmaceutical combination for treating, alleviating or delaying progression of the symptoms of a demyelinating disease.

13. Use of an S1P receptor agonist for the preparation of a medicament for treating, alleviating or delaying the progression of optic neuritis.

Electronic Acknowledgement Receipt

EFS ID:	11081301
Application Number:	13149468
International Application Number:	
Confirmation Number:	1536
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Customer Number:	1095
Filer:	Karen DeBenedictis/Denise Cooper
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		50279_CNT_IDS.pdf	187681 e51219ae72e6bab935f8843029a7d76fdf1c6e3a	yes	3

	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Transmittal Letter		1	1	
	Information Disclosure Statement (IDS) Form (SB08)		2	3	
Warnings:					
Information:					
2	Foreign Reference	WO03099192.pdf	1892887 6e8701da61ce0c497d932370412163ebcbd223ac	no	45
Warnings:					
Information:					
3	Foreign Reference	WO03097028.pdf	2444945 f9b23b57d40b545fe0e843317fb7709c0d3c3f03	no	49
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4	Foreign Reference	WO04050073.pdf	1561221 a169c1a9bb2b97cb26c029a26b5700da62a3f608	no	24
Warnings:					
Information:					
5	Foreign Reference	WO05123104.pdf	1096874 deb5c789fb5712e1e2ac5f334d19ac1d531c9b2e	no	19
Warnings:					
Information:					
6	Non Patent Literature	LaMontagne.pdf	21746088 fbf1bed67337d01daf1e32c89ebfce924149795e	no	11
Warnings:					
Information:					
7	Non Patent Literature	Ho.pdf	1299531 cb10de653d6061c98ef4a1865c0a39a3f08acfbb	no	10
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8	Non Patent Literature	Virely.pdf	158514 9034b9d785d0b1ded247548c811e64f2d5ad5984	no	12
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9	Non Patent Literature	Hla2004.pdf	102072 b2772ee76e115a6f8f7e322b4a884e520a607fa9	no	8
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10	Non Patent Literature	Kappos.pdf	39752 adbe47f0da4060fa8a3317e89eb12523346c28f8	no	2
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11	Foreign Reference	WO04028521.pdf	3033930 3f3459886dc5c388f4f45bf0449f5e5146f44839	no	29
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			Total Files Size (in bytes):	33563495	

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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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO: 13/149468
FILED: May 31, 2011
FOR: DOSAGE REGIMEN OF AN S1P RECEPTOR AGONIST

Art Unit: 1629
Examiner:

MS: Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Sir:

This paper is being filed:

☒ supplemental to the Information Disclosure Statement filed .

☒ before the mailing date of a first Office Action on the merits, and so under 37 C.F.R. §1.97(b)(3) no fees are required.

If a fee is deemed to be required, the Commissioner is hereby authorized to charge such fee to Deposit Account No. 19-0134 in the name of Novartis.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO/SB/08A/B.

☒ Copies of the references are enclosed herewith.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO/SB/08A/B form(s).

Respectfully submitted,

Karen DeBenedictis
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Date: 9/29/11

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/149,468

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	330
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	540
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	220
TOTAL CLAIMS (37 CFR 1.16(i))	11 minus 20 =	*			OR	x 52 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*				x 220 =	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 $270 ($135 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))							390
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1480

APPLICATION AS AMENDED - PART II

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

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

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

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

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
13149468	

Applicant(s) Peter Hiestand

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
1			1			
2			1			
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Total Indep	0		3		0	
Total Depend	0	↵	8	↵	0	↵
Total Claims	0		11		0	

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	Indep	Depend	Indep	Depend	Indep	Depend
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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/149,468	05/31/2011	1629	1480	PAT050279-US-CNT	10	3

CONFIRMATION NO. 1536

1095
NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

FILING RECEIPT

OC000000048231974

Date Mailed: 06/20/2011

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**

Applicant(s)

Peter C. Hiestand, Allschwil, SWITZERLAND;
Christian Schnell, Hesingue, FRANCE;

Power of Attorney: The patent practitioners associated with Customer Number 001095

Domestic Priority data as claimed by applicant

This application is a CON of 12/303,765 12/08/2008 ABN
which is a 371 of PCT/EP2007/005597 06/25/2007

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
UNITED KINGDOM 0612721.1 06/27/2006

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If Required, Foreign Filing License Granted: 06/15/2011

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/149,468**

Projected Publication Date: 09/29/2011

Non-Publication Request: No

Early Publication Request: No

Title

S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS

Preliminary Class

514

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	2

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
		WO 2006/058316	06-01-2006			☐
		WO 2004/113330	12-29-2004			☐
						☐
						☐
						☐
						☐

Examiner Signature		**Date Considered**	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw a line through citation if not in conformance and not considered. Include copy of this form with the next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kind Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

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(Use as many sheets as necessary)

Sheet	2	of	2

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	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[2]
		Brinkmann, Volker et al., "The Immune Modulator FTY720 Targets Sphingosine 1-Phosphate Receptors", The Journal of Biological Chemistry, Vol. 277, No. 24, Issue of June 14, pp. 21453-21457, (2002).	☐
		Miller et al., Neurol, & Neurosci. Reports, (September, 2010), 1095), pp. 397-406	☐
		Hla, T., FASEB Journal, (March 6, 2006), 20(4), part 1, A20.	☐
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Examiner Signature		**Date Considered**	

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Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Filer:	Karen DeBenedictis/Denise Cooper
Attorney Docket Number:	PAT050279-US-CNT

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	330	330
Utility Search Fee	1111	1	540	540
Utility Examination Fee	1311	1	220	220
Pages:				
Claims:				
Multiple dependent claims	1203	1	390	390
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**1480**

Electronic Acknowledgement Receipt

EFS ID:	10199863
Application Number:	13149468
International Application Number:	
Confirmation Number:	1536
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Customer Number:	01095
Filer:	Karen DeBenedictis/Denise Cooper
Filer Authorized By:	Karen DeBenedictis
Attorney Docket Number:	PAT050279-US-CNT
Receipt Date:	31-MAY-2011
Filing Date:	
Time Stamp:	17:48:14
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1480
RAM confirmation Number	5139
Deposit Account	190134
Authorized User	

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Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		50279_CNT_PCTAPPLN.pdf	730979 4e4ab92ca95a3ecdd01f27f624483de675c7bcff	yes	17

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Specification	1	15
Claims	16	17

Warnings:

Information:

2		50279_CNT_Appln.pdf	628946 500ee6065f08db80cf63315c2088588988f6a804	yes	17

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Transmittal of New Application	1	2
Application Data Sheet	3	5
Oath or Declaration filed	6	8
Preliminary Amendment	9	14
Transmittal Letter	15	15
Information Disclosure Statement (IDS) Filed (SB/08)	16	17

Warnings:

Information:

3	Fee Worksheet (PTO-875)	fee-info.pdf	36539 6e00ebf527b026a0d669945735d9d1765f044e1b	no	2

Warnings:

Information:

Total Files Size (in bytes):	1396464

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

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New International Application Filed with the USPTO as a Receiving Office

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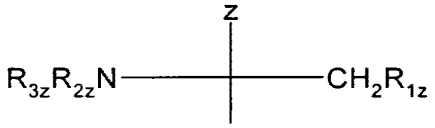
Organic Compounds

The present invention relates to the use of an S1P receptor modulator in the treatment or prevention of neo-angiogenesis associated with a demyelinating disease, e.g. multiple sclerosis.

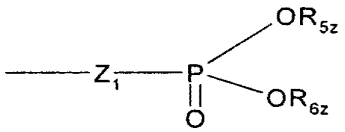
S1 P receptor modulators are typically sphingosine analogues, such as 2-substituted 2-amino- propane-1,3-diol or 2-amino-propanol derivatives, e. g. a compound comprising a group of formula X .

Sphingosine-1 phosphate (hereinafter "S1P") is a natural serum lipid. Presently there are eight known S1P receptors, namely S1P1 to S1P8. S1 P receptor modulators are typically sphingosine analogues, such as 2-substituted 2-amino- propane-1,3-diol or 2-amino-propanol derivatives, e. g. a compound comprising a group of formula X



(X)

wherein Z is H, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, phenyl, phenyl substituted by OH, C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH, or CH_2-R_{4z} wherein R_{4z} is OH, acyloxy or a residue of formula (a)



(a)

wherein Z_1 is a direct bond or O, preferably O;

each of R_{5z} and R_{6z}, independently, is H, or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms;

R_{1z} is OH, acyloxy or a residue of formula (a); and each of R_{2z} and R_{3z} independently, is H, C_{1-4}alkyl or acyl.

Group of formula X is a functional group attached as a terminal group to a moiety which may be hydrophilic or lipophilic and comprise one or more aliphatic, alicyclic, aromatic and/or heterocyclic residues, to the extent that the resulting molecule wherein at least one of Z and

R_{1z} is or comprises a residue of formula (a), signals as an agonist at one of more sphingosine-1-phosphate receptor.

S1P receptor modulators are compounds which signal as agonists at one or more sphingosine-1 phosphate receptors, e.g. S1P1 to S1P8. Agonist binding to a S1P receptor may e.g. result in dissociation of intracellular heterotrimeric G-proteins into Gα-GTP and Gβγ-GTP, and/or increased phosphorylation of the agonist-occupied receptor and activation of downstream signaling pathways/kinases.

The binding affinity of S1P receptor modulators to individual human S1P receptors may be determined in following assay:
S1P receptor modulator activities of compounds are tested on the human S1P receptors $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$ and $S1P_5$. Functional receptor activation is assessed by quantifying compound induced GTP [γ-^{35}S] binding to membrane protein prepared from transfected CHO or RH7777 cells stably expressing the appropriate human S1P receptor. The assay technology used is SPA (scintillation proximity based assay). Briefly, DMSO dissolved compounds are serially diluted and added to SPA- bead (Amersham-Pharmacia) immobilised S1P receptor expressing membrane protein (10-20μg/well) in the presence of 50 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$, 10 μM GDP, 0.1% fat free BSA and 0.2 nM GTP [γ-^{35}S] (1200 Ci/mmol). After incubation in 96 well microtiterplates at RT for 120 min, unbound GTP [γ-^{35}S] is separated by a centrifugation step. Luminescence of SPA beads triggered by membrane bound GTP [γ-^{35}S] is quantified with a TOPcount plate reader (Packard). EC_{50}s are calculated using standard curve fitting software. In this assay, the S1P receptor modulators preferably have a binding affinity to S1P receptor <50 nM.

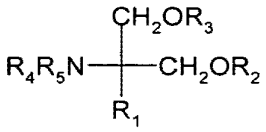
Preferred S1P receptor modulators are e.g. compounds which in addition to their S1P binding properties also have accelerating lymphocyte homing properties, e.g. compounds which elicit a lymphopenia resulting from a re-distribution, preferably reversible, of lymphocytes from circulation to secondary lymphatic tissue, without evoking a generalized immunosuppression. Naïve cells are sequestered; CD4 and CD8 T-cells and B-cells from the blood are stimulated to migrate into lymph nodes (LN) and Peyer's patches (PP).

The lymphocyte homing property may be measured in following Blood Lymphocyte Depletion assay:
A S1P receptor modulator or the vehicle is administered orally by gavage to rats. Tail blood for hematological monitoring is obtained on day –1 to give the baseline individual values, and at 2, 6, 24, 48 and 72 hours after application. In this assay, the S1P receptor agonist or

modulator depletes peripheral blood lymphocytes, e.g. by 50%, when administered at a dose of e.g. < 20 mg/kg.

Examples of appropriate S1P receptor modulators are, for example:

- Compounds as disclosed in EP627406A1, e.g. a compound of formula I



I

wherein R_1 is a straight- or branched (C_{12-22})chain

- which may have in the chain a bond or a hetero atom selected from a double bond, a triple bond, O, S, NR_6, wherein R_6 is H, C_{1-4}alkyl, aryl-C_{1-4}alkyl, acyl or (C_{1-4}alkoxy)carbonyl, and carbonyl, and/or
    - which may have as a substituent C_{1-4}alkoxy, C_{2-4}alkenyloxy, C_{2-4}alkynyloxy, arylC_{1-4}alkyl-oxy, acyl, C_{1-4}alkylamino, C_{1-4}alkylthio, acylamino, (C_{1-4}alkoxy)carbonyl, (C_{1-4}alkoxy)-carbonylamino, acyloxy, (C_{1-4}alkyl)carbamoyl, nitro, halogen, amino, hydroxyimino, hydroxy or carboxy; or

R_1 is

- a phenylalkyl wherein alkyl is a straight- or branched (C_{6-20})carbon chain; or
- a phenylalkyl wherein alkyl is a straight- or branched (C_{1-30})carbon chain wherein said phenylalkyl is substituted by
- a straight- or branched (C_{6-20})carbon chain optionally substituted by halogen,
- a straight- or branched (C_{6-20})alkoxy chain optionally substitued by halogen,
- a straight- or branched (C_{6-20})alkenyloxy,
- phenyl-C_{1-14}alkoxy, halophenyl-C_{1-4}alkoxy, phenyl-C_{1-14}alkoxy-C_{1-14}alkyl, phenoxy-C_{1-4}alkoxy or phenoxy-C_{1-4}alkyl,
- cycloalkylalkyl substituted by C_{6-20}alkyl,
- heteroarylalkyl substituted by C_{6-20}alkyl,
- heterocyclic C_{6-20}alkyl or
- heterocyclic alkyl substituted by C_{2-20}alkyl,

and wherein

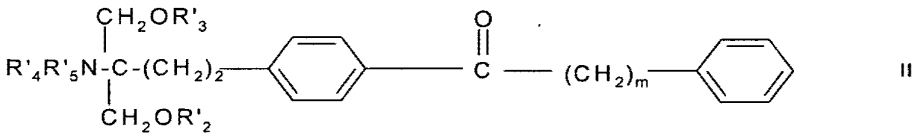
the alkyl moiety may have

- in the carbon chain, a bond or a heteroatom selected from a double bond, a triple bond, O, S, sulfinyl, sulfonyl, or NR_6, wherein R_6 is as defined above, and
- as a substituent C_{1-4}alkoxy, C_{2-4}alkenyloxy, C_{2-4}alkynyloxy, arylC_{1-4}alkyloxy, acyl, C_{1-4}alkylamino, C_{1-4}alkylthio, acylamino, (C_{1-4}alkoxy)carbonyl, (C_{1-4}alkoxy)carbonylamino, acyloxy, (C_{1-4}alkyl)carbamoyl, nitro, halogen, amino, hydroxy or carboxy, and

each of R_2, R_3, R_4 and R_5, independently, is H, C_{1-4} alkyl or acyl

or a pharmaceutically acceptable salt or hydrate thereof;

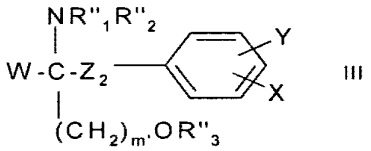
- Compounds as disclosed in EP 1002792A1, e.g. a compound of formula II



II

wherein m is 1 to 9 and each of R'_2, R'_3, R'_4 and R'_5, independently, is H, C_{1-6}alkyl or acyl, or a pharmaceutically acceptable salt or hydrate thereof;

- Compounds as disclosed in EP0778263 A1, e.g. a compound of formula III



III

wherein W is H; C_{1-6}alkyl, C_{2-6}alkenyl or C_{2-6}alkynyl; unsubstituted or by OH substituted phenyl; $R''_4O(CH_2)_n$; or C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH;

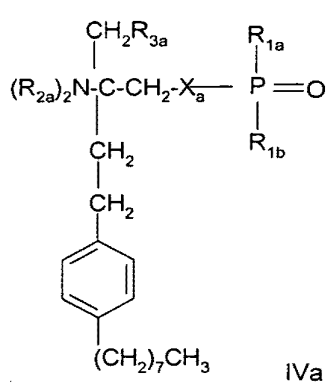
X is H or unsubstituted or substituted straight chain alkyl having a number p of carbon atoms or unsubstituted or substituted straight chain alkoxy having a number (p-1) of carbon atoms, e.g. substituted by 1 to 3 substitutents selected from the group consisting of C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyloxy, amino, C_{1-6}alkylamino, acylamino, oxo, haloC_{1-6}alkyl, halogen, unsubstituted phenyl and phenyl substituted by 1 to 3 substituents selected from the group consisting of C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl and halogen; Y is H, C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl or halogen, Z_2 is a single bond or a straight chain alkylene having a number or carbon atoms of q,

each of p and q, independently, is an integer of 1 to 20, with the proviso of $6 \leq p+q \leq 23$, m' is 1, 2 or 3, n is 2 or 3,

each of R''_1, R''_2, R''_3 and R''_4, independently, is H, C_{1-4}alkyl or acyl,

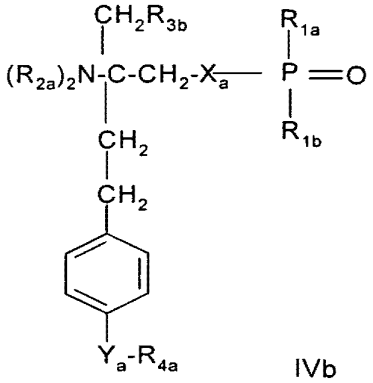
or a pharmaceutically acceptable salt or hydrate thereof,

- Compounds as disclosed in WO02/18395, e.g. a compound of formula IVa or IVb



IVa

or

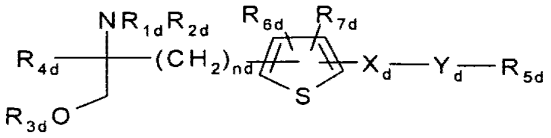


IVb

wherein X_a is O, S, NR_{1s} or a group $-(CH_2)_{na}-$, which group is unsubstituted or substituted by 1 to 4 halogen; n_a is 1 or 2, R_{1s} is H or (C_{1-4})alkyl, which alkyl is unsubstituted or substituted by halogen; R_{1a} is H, OH, (C_{1-4})alkyl or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by 1 to 3 halogen; R_{1b} is H, OH or (C_{1-4})alkyl, wherein alkyl is unsubstituted or substituted by halogen; each R_{2a} is independently selected from H or (C_{1-4})alkyl, which alkyl is unsubstituted or substitued by halogen; R_{3a} is H, OH, halogen or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; and R_{3b} is H, OH, halogen, (C_{1-4})alkyl wherein alkyl is unsubstituted or substituted by hydroxy, or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; Y_a is $-CH_2-$, -C(O)-, -CH(OH)-, -C(=NOH)-, O or S, and R_{4a} is (C_{4-14})alkyl or (C_{4-14})alkenyl;

or a pharmaceutically acceptable salt or hydrate thereof;

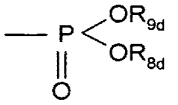
- Compounds as disclosed in WO02/06268Al, e.g. a compound of formula V



V

wherein each of R_{1d} and R_{2d}, independently, is H or an amino-protecting group;

R_{3d} is hydrogen, a hydroxy-protecting group or a residue of formula



R_{4d} is C_{1-4}alkyl;

n_d is an integer of 1 to 6;

X_d is ethylene, vinylene, ethynylene, a group having a formula – D-CH_2- (wherein D is carbonyl, – CH(OH)-, O, S or N), aryl or aryl substituted by up to three substitutents selected from group a as defined hereinafter;

Y_d is single bond, C_{1-10}alkylene, C_{1-10}alkylene which is substituted by up to three substitutents selected from groups a and b, C_{1-10}alkylene having O or S in the middle or end of the carbon chain, or C_{1-10}alkylene having O or S in the middle or end of the carbon chain which is substituted by up to three substituents selected from groups a and b;

R_{5d} is hydrogen, C_{3-6}cycloalkyl, aryl, heterocyclic group, C_{3-6}cycloalkyl substituted by up to three substituents selected from groups a and b, aryl substituted by up to three substituents selected from groups a and b, or heterocyclic group substituted by up to three substituents selected from groups a and b;

each of R_{6d} and R_{7d}, independently, is H or a substituent selected from group a;

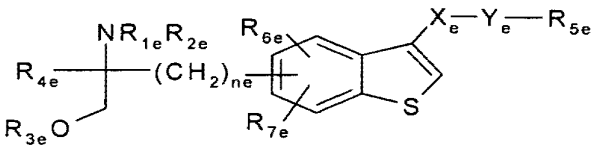
each of R_{8d} and R_{9d}, independently, is H or C_{1-4}alkyl optionally substituted by halogen;

<group a > is halogen, lower alkyl, halogeno lower alkyl, lower alkoxy, lower alkylthio, carboxyl, lower alkoxycarbonyl, hydroxy, lower aliphatic acyl, amino, mono-lower alkylamino, di-C_{1-4}alkylamino, acylamino, cyano or nitro; and

<group b > is C_{3-6}cycloalkyl, aryl or heterocyclic group, each being optionally substituted by up to three substituents selected from group a;

with the proviso that when R_{5d} is hydrogen, Y_d is a either a single bond or linear C_{1-10} alkylene, or a pharmacologically acceptable salt, ester or hydrate thereof;

-Compounds as disclosed in JP-14316985 (JP2002316985), e.g. a compound of formula VI

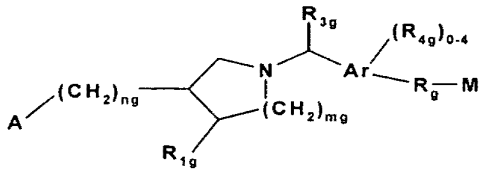


VI

wherein R_{1e},R_{2e},R_{3e},R_{4e},R_{5e},R_{6e},R_{7e}, n_e, X_e and Y_e are as disclosed in JP-14316985;

or a pharmacologically acceptable salt, ester or hydrate thereof;

-Compounds as disclosed in WO03/062252A1, e.g. a compound of formula VII

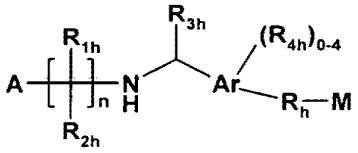


VII

wherein

Ar is phenyl or naphthyl; each of m_g and n_g independently is 0 or 1; A is selected from COOH, PO_3H_2, PO_2H, SO_3H, $PO(C_{1-3}alkyl)OH$ and 1*H*-tetrazol-5-yl; each of R_{1g} and R_{2g} independently is H, halogen, OH, COOH or C_{1-4}alkyl optionally substituted by halogen; R_{3g} is H or C_{1-4}alkyl optionally substituted by halogen or OH; each R_{4g} independently is halogen, or optionally halogen substituted C_{1-4}alkyl or C_{1-3}alkoxy; and each of R_g and M has one of the significances as indicated for B and C, respectively, in WO03/062252A1;
or a pharmacologically acceptable salt, solvate or hydrate thereof;

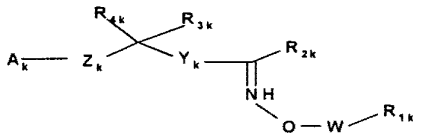
-Compounds as disclosed in WO 03/062248A2, e.g. a compound of formula VIII



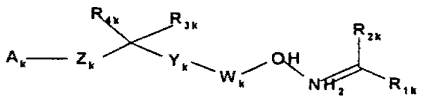
VIII

wherein Ar is phenyl or naphthyl; n is 2,3 or 4; A is COOH, 1*H*-tetrazol-5-yl, PO_3H_2, PO_2H_2, -SO_3H or $PO(R_{5h})OH$ wherein R_{5h} is selected from C_{1-4}alkyl, hydroxyC_{1-4}alkyl, phenyl, -CO-C_{1-3}alkoxy and –CH(OH)-phenyl wherein said phenyl or phenyl moiety is optionally substituted; each of R_{1h} and R_{2h} independently is H, halogen, OH, COOH, or optionally halogeno substituted C_{1-6}alkyl or phenyl; R_{3h} is H or C_{1-4}alkyl optionally substituted by halogen and/ OH; each R_{4h} independently is halogeno, OH, COOH, C_{1-4}alkyl, $S(O)_{0,1\ or2}C_{1-3}$alkyl, C_{1-3}alkoxy, C_{3-6}cycloalkoxy, aryl or aralkoxy, wherein the alkyl portions may optionally be substituted by 1-3 halogens; and each of R_h and M has one of the significances as indicated for B and C, respectively, in WO03/062248A2
or a pharmacologically acceptable salt, solvate or hydrate thereof.

- Compounds as disclosed in WO 04/103306A, WO 05/000833, WO 05/103309 or WO 05/113330, e.g. compounds of formula IXa or IXb



IXa



IXb

wherein

A_k is $COOR_{5k}$, $OPO(OR_{5k})_2$, $PO(OR_{5k})_2$, SO_2OR_{5k}, $POR_{5k}OR_{5k}$ or 1*H*-tetrazol-5-yl, R_{5k} being H or C_{1-6}alkyl;

W_k is a bond, C_{1-3}alkylene or C_{2-3}alkenylene;

Y_k is C_{6-10}aryl or C_{3-9}heteroaryl, optionally substituted by 1 to 3 radicals selected from halogene, OH, NO_2, C_{1-6}alkyl, C_{1-6}alkoxy; halo-substituted C_{1-6}alkyl and halo-substituted C_{1-6}alkoxy;

Z_k is a heterocyclic group as indicated in WO 04/103306A, e.g. azetidine;

R_{1k} is C_{6-10}aryl or C_{3-9}heteroaryl, optionally substituted by C_{1-6}alkyl, C_{6-10}aryl, C_{6-10}arylC_{1-4}alkyl, C_{3-9}heteroaryl, C_{3-9}heteroarylC_{1-4}alkyl, C_{3-8}cycloalkyl, C_{3-8}cycloalkylC_{1-4}alkyl, C_{3-8}heterocycloalkyl or C_{3-8}heterocycloalkylC_{1-4}alkyl; wherein any aryl, heteroaryl, cycloalkyl or heterocycloalkyl of R_{1k} may be substituted by 1 to 5 groups selected from halogen, C_{1-6}alkyl, C_{1-6}alkoxy and halo substituted-C_{1-6}alkyl or -C_{1-6}alkoxy;

R_{2k} is H, C_{1-6}alkyl, halo substituted C_{1-6}alkyl, C_{2-6}alkenyl or C_{2-6}alkynyl: and

each of R_{3k} or R_{4k}, independently, is H, halogen, OH, C_{1-6}alkyl, C_{1-6}alkoxy or halo substituted C_{1-6}alkyl or C_{1-6}alkoxy;

and the N-oxide derivatives thereof or prodrugs thereof,

or a pharmacologically acceptable salt, solvate or hydrate thereof.

The compounds of formulae I to IXb may exist in free or salt form. Examples of pharmaceutically acceptable salts of the compounds of the formulae I to VI include salts with inorganic acids, such as hydrochloride, hydrobromide and sulfate, salts with organic acids, such as acetate, fumarate, maleate, benzoate, citrate, malate, methanesulfonate and benzenesulfonate salts, or, when appropriate, salts with metals such as sodium, potassium, calcium and aluminium, salts with amines, such as triethylamine and salts with dibasic amino acids, such as lysine. The compounds and salts of the combination of the present invention encompass hydrate and solvate forms.

Acyl as indicated above may be a residue R_y-CO- wherein R_y is C_{1-6}alkyl, C_{3-6}cycloalkyl, phenyl or phenyl-C_{1-4}alkyl. Unless otherwise stated, alkyl, alkoxy, alkenyl or alkynyl may be straight or branched.

Aryl may be phenyl or naphthyl, preferably phenyl.

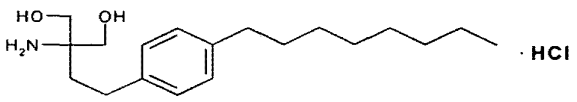
When in the compounds of formula I the carbon chain as R_1 is substituted, it is preferably substituted by halogen, nitro, amino, hydroxy or carboxy. When the carbon chain is interrupted by an optionally substituted phenylene, the carbon chain is preferably unsubstituted. When the phenylene moiety is substituted, it is preferably substituted by halogen, nitro, amino, methoxy, hydroxy or carboxy.

Preferred compounds of formula I are those wherein R_1 is C_{13-20}alkyl, optionally substituted by nitro, halogen, amino, hydroxy or carboxy, and, more preferably those wherein R_1 is phenylalkyl substituted by C_{6-14}-alkyl chain optionally substituted by halogen and the alkyl moiety is a C_{1-6}alkyl optionally substituted by hydroxy. More preferably, R_1 is phenyl-C_{1-6}alkyl substituted on the phenyl by a straight or branched, preferably straight, C_{6-14}alkyl chain. The C_{6-14}alkyl chain may be in ortho, meta or para, preferably in para.

Preferably each of R_2 to R_5 is H.

In the above formula of V "heterocyclic group" represents a 5- to 7 membered heterocyclic group having 1 to 3 heteroatoms selected from S, O and N. Examples of such heterocyclic groups include the heteroaryl groups indicated above, and heterocyclic compounds corresponding to partially or completely hydrogenated heteroaryl groups, e.g. furyl, thienyl, pyrrolyl, azepinyl, pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, 1,2,3-oxadiazolyl, triazolyl, tetrazolyl, thiadiazolyl, pyranyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, tetrahydropyranyl, morpholinyl, thiomorpholinyl, pyrrolidinyl, pyrrolyl, imidazolidinyl, pyrazolidinyl, piperidinyl, piperazinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl or pyrazolidinyl. Preferred heterocyclic groups are 5-or 6-membered heteroaryl groups and the most preferred heteocyclic group is a morpholinyl, thiomorpholinyl or piperidinyl group.

A preferred compound of formula I is 2-amino-2-tetradecyl-1,3-propanediol. A particularly preferred S1P receptor agonist of formula I is FTY720, i.e. 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol in free form or in a pharmaceutically acceptable salt form (referred to hereinafter as Compound A), e.g. the hydrochloride salt, as shown:



A preferred compound of formula II is the one wherein each of R'_2 to R'_5 is H and m is 4, i.e. 2-amino-2-{2-[4-(1-oxo-5-phenylpentyl)phenyl]ethyl}propane-1,3-diol, in free form or in pharmaceutically acceptable salt form (referred to hereinafter as Compound B), e.g the hydrochloride.

A preferred compound of formula III is the one wherein W is CH_3, each of R''_1 to R''_3 is H, Z_2 is ethylene, X is heptyloxy and Y is H, i.e. 2-amino-4-(4-heptyloxyphenyl)-2-methyl-butanol, in free form or in pharmaceutically acceptable salt form (referred to hereinafter as Compound C), e.g. the hydrochloride. The R-enantiomer is particularly preferred.

Compounds may e in phosphorylated form. A preferred compound of formula IVa is the FTY720-phosphate (R_{2a} is H, R_{3a} is OH, X_a is O, R_{1a} and R_{1b} are OH). A preferred compound of formula IVb is the Compound C-phosphate (R_{2a} is H, R_{3b} is OH, X_a is O, R_{1a} and R_{1b} are OH, Y_a is O and R_{4a} is heptyl). A preferred compound of formula V is Compound B-phosphate.

A preferred compound of formula VI is (2R)-2-amino-4-[3-(4-cyclohexyloxybutyl)-benzo[b]thien-6-yl]-2-methylbutan-1-ol.

A preferred compound of formula IXa is e.g. 1-{4-[1-(4-cyclohexyl-3-trifluoromethyl-benzyloxyimino)-ethyl]-2-ethyl-benzyl}-azetidine-3-carboxylic acid, or a prodrug thereof.

S1P receptor agonists or modulators are known as having immunosuppressive properties or anti-angiogenic properties in the treatment of tumors, e.g. as disclosed in EP627406A1, WO 04/103306, WO 05/000833, WO 05/103309, WO 05/113330 or WO 03/097028.

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system with chronic inflammatory demyelination leading to progressive decline of motor and sensory functions and permanent disability. The therapy of multiple sclerosis is only partially effective, and in most cases only offers a short delay in disease progression despite anti-inflammatory and immunosuppressive treatment. Accordingly, there is a need for agents which are effective in the inhibition or treatment of demyelinating diseases, e.g. multiple sclerosis or Guillain-Barré syndrome, including reduction of, alleviation of, stabilization of or relief from the symptoms which affect the organism.

Characteristic pathological features of demyelinating diseases include inflammation, demyelination and axonal and oligodendrocyte loss. In addition lesions can also have a significant vascular component. A firm link has recently been established between chronic inflammation and angiogenesis and neovascularization seems to have a significant role in the progression of disease.

It has now been found that S1P receptor modulators have an inhibitory effect on neo-angiogenesis associated with demyelinating diseases, e.g. MS.

In a series of further specific or alternative embodiments, the present invention provides:

1.1 A method for preventing, inhibiting or treating neo-angiogenesis associated with a demyelinating disease, e.g. MS, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to IXb.

1.2 A method for alleviating or delaying progression of the symptoms of a demyelinating disease, e.g. multiple sclerosis or Guillain-Barré syndrome, in a subject in need thereof, in which method neo-angiogenesis associated with said disease is prevented or inhibited, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to IXb.

1.3 A method for reducing or preventing or alleviating relapses in a demyelinating disease, e.g. multiple sclerosis or Guillain-Barré syndrome, in a subject in need thereof, in which method neo-angiogenesis associated with said disease is prevented or inhibited, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to IXb.

1.4 A method for slowing progression of a demyelinating disease, e.g. multiple sclerosis or Guillain-Barré syndrome, in a subject being in a relapsing-remitting phase of the disease, in which method neo-angiogenesis associated with said disease is prevented or inhibited, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to XIb.

1.5 A method as indicated above, wherein the S1P receptor modulator is administered intermittently.

For example, the S1P receptor modulator may be administered to the subject every 2nd or 3rd day or once a week.

2. A pharmaceutical composition for use in any one of the methods 1.1 to 1.5, comprising an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined hereinabove, together with one or more pharmaceutically acceptable diluents or carriers therefor.

3. An S1P receptor modulator, e.g a compound of formula I to IXb as defined herein above, for use in any one of the methods 1.1 to 1.5.

4 An S1P receptor modulator, e.g. a compound of formulae I to IXb as defined herein above, for use in the preparation of a medicament for use in any one of the methods 1.1 to 1.5.

Clinicians usually categorize patients having MS into four types of disease patterns:

- *Relapsing-remitting* (RR-MS): Discrete motor, sensory, cerebellar or visual attacks that occur over 1-2 weeks and often resolve over 1-2 months. Some patients accrue disability with each episode, yet remain clinically stable between relapses. About 85% of patients initially experience the RR form of MS, but within 10 years about half will develop the secondary progressive form.
- *Secondary-progressive* (SP-MS): Initially RR followed by gradually increasing disability, with or without relapses. Major irreversible disabilities appear most often during SP.
- *Primary-progressive* (PP-MS): Progression disease course from onset without any relapses or remissions, affecting about 15% of MS patients.
- *Progressive-relapsing* (PR-MS): Progressive disease from onset with clear acute relapses; periods between relapses characterized by continuing progression.

Accordingly, the S1P receptor modulators, e.g. a compound of formulae I to IXb as defined hereinabove, may be useful in the treatment of one or more of *Relapsing-remitting* (RR-MS), *Secondary-progressive* (SP-MS), *Primary-progressive* (PP-MS) and *Progressive-relapsing* (PR-MS).

In particular, the S1P receptor modulators as described herein, e.g. FTY720, i.e. 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-dio, are useful for treating PP-MS.

Utility of the S1P receptor modulators, e.g. the S1P receptor modulators comprising a group of formula X, in preventing or treating neo-angiogenesis associated with a demyelinating disease as hereinabove specified, may be demonstrated in animal test methods as well as in clinic, for example in accordance with the methods hereinafter described.

In vivo: Relapsing Experimental Autoimmune Encephalomyelitis (EAE)

Disease is induced in female Lewis rats by immunization with guinea pig spinal cord tissue emulsified in complete Freund's adjuvant. This results in an acute disease within 11 days, followed by an almost complete remission around day 16 and a relapse at around days 26.

On day 26 rats are thoracectomized after having been deeply anesthetized with Isoflurane (3%, 20 L / min) and perfused through the left ventricle of the heart. The left ventricle is punctured with a 19 gauge needle from a winged infusion set (SV-19BLK; Termudo, Elkton, MD), which is connected to an airtight pressurized syringe containing the rinsing solution (NaCl 0.9% with 250,000 U/l heparin at 35°C). The right atrium is punctured to provide outflow, and the perfusate is infused under a precise controlled pressure of 120 mm Hg. The perfusion is continued for 5 min (at a constant rate of 20 ml/min) followed by a pre-fixation solution (2% performaldehyde in PBS at 35°C). Finally, up to 30 ml of polyurethane resin (PUI14; Vasqtec, Zürich, Switzerland) is infused at the same rate. After 48 h, the resin-filled brain and spinal cord are excised from the animal and the soft tissue removed by maceration in 7.5% KOH during 24 hr at 50°C. The casts are then thoroughly cleaned with and stored in distilled water before drying by lyophilization. These vascular casts are quantitated using micro computer tomography.

In this assay, a S1P1 receptor modulator, e.g. Compound A significantly blocks disease-associated neo-angiogenesis when administered to the animals at a dose of from 0.1 to 20 mg/kg p.o. For example, Compound A, in the hydrochloride salt form, fully blocks disease-associated angiogenesis and completely inhibits the relapse phases when administered daily at a dose of 0.3 mg/kg p.o. The same effect is obtained when Compound A, in the hydrochloride salt form, is administered p.o. at 0.3 mg/kg every 2nd or 3rd day or once a week.

C. Clinical Trial

Investigation of clinical benefit of a S1P receptor agonist, e.g. a compound of formula I, e.g. Compound A.

20 patients with relapsing-remitting MS receive said compound at a daily dosage of 0.5, 1.25 or 2.5 mg p.o. The general clinical state of the patient is investigated weekly by physical and laboratory examination. Disease state and changes in disease progression are assessed every 2 months by radiological examination (MRI) and physical examination. Initially patients receive treatment for 2 to 6 months. Thereafter, they remain on treatment for as long as their disease does not progress and the drug is satisfactorily tolerated.

Main variables for evaluation: Safety (adverse events), standard serum biochemistry and hematology, magnetic resonance imaging (MRI).

Daily dosages required in practicing the method of the present invention when a S1P receptor modulator alone is used will vary depending upon, for example, the compound used, the host, the mode of administration and the severity of the condition to be treated. A preferred daily dosage range is about from 0.1 to 100 mg as a single dose or in divided

doses. Suitable daily dosages for patients are on the order of from e.g. 0.1 to 50 mg p.o. The S1P receptor modulator may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions, nasally, pulmonary (by inhalation) or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration comprise from ca. 0.1 to 30 mg, usually 0.25 to 30 mg S1P receptor modulator, together with one or more pharmaceutically acceptable diluents or carriers therefore. As already mentioned, the S1Preceptor modulator, e.g. Compound A, may alternatively be administered intermittently, e.g. at a dose of 0.5 to 30 mg every other day or once a week.

According to another embodiment of the invention, the S1P receptor modulator may be administered as the sole active ingredient or in conjunction with, e.g. as an adjuvant to, a VEGF-receptor antagonist.

Examples of suitable VEGF-receptor antagonist include e.g. compounds, proteins or antibodies which inhibit the VEGF receptor tyrosine kinase, inhibit a VEGF receptor or bind to VEGF, and are e.g. in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 98/35958, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or a pharmaceutically acceptable salt thereof, e.g. the succinate, in WO 00/27820, e.g. a N-aryl(thio) anthranilic acid amide derivative e.g. 2-[(4-pyridyl)methyl]amino-N-[3-methoxy-5-(trifluoromethyl)phenyl]benzamide or 2-[(1-oxido-4-pyridyl)methyl]amino-N-[3-trifluoromethylphenyl]benzamide, or in WO 00/09495, WO 00/59509, WO 98/11223, WO 00/27819, WO 01/55114, WO 01/58899 and EP 0 769 947; those as described by M. Prewett et al in Cancer Research 59 (1999) 5209-5218, by F. Yuan et al in Proc. Natl. Acad. Sci. USA, vol. 93, pp. 14765-14770, Dec. 1996, by Z. Zhu et al in Cancer Res. 58, 1998, 3209-3214, and by J. Mordenti et al in Toxicologic Pathology, Vol. 27, no. 1, pp 14-21, 1999; in WO 00/37502 and WO 94/10202; Angiostatin™, described by M. S. O'Reilly et al, Cell 79, 1994, 315-328; Endostatin™, described by M. S. O'Reilly et al, Cell 88, 1997, 277-285; anthranilic acid amides; ZD4190; ZD6474; SU5416; SU6668; or anti-VEGF antibodies or anti-VEGF receptor antibodies,e.g. RhuMab.

4-Pyridylmethyl-phthalazine derivatives are e.g. preferred inhibitors of VEGF receptor tyrosine kinase. Such derivatives and their preparation, pharmaceutical formulations thereof and methods of making such compounds are described in WO00/59509, EP02/04892, WO01/10859 and, in particular, in U.S. Patent No. 6,258,812, which are here incorporated by reference.

Where the S1P receptor modulator is administered in conjunction with a VEGF-receptor antagonist, dosages of the co-administered VEGF-receptor agonist will of course vary depending on the type of co-drug employed, e.g. whether it is a steroid or a calcineurin inhibitor, on the specific drug employed, on the condition being treated and so forth. In accordance with the foregoing the present invention provides in a yet further aspect:

5. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective non-toxic amount of a S1P receptor modulator and a VEGF-receptor antagonist, e.g. as indicated above.

6. A pharmaceutical combination, e.g. a kit, comprising a) a first agent which is a S1P receptor modulator as disclosed herein, in free form or in pharmaceutically acceptable salt form, and b) a VEGF-receptor antagonist, e.g. as indicated above. The kit may comprise instructions for its administration.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

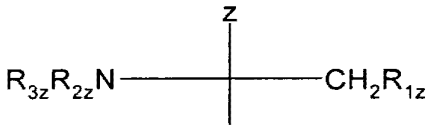
The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a S1P receptor modulator and a VEGF-receptor antagonist, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a S1P receptor modulator and a VEGF-receptor antagonist, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the 2 compounds in the body of the patient.

CLAIMS

1. Use of an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined herein above, in the preparation of a medicament for preventing, inhibiting or treating neo-angiogenesis associated with a demyelinating disease, e.g. multiple sclerosis.

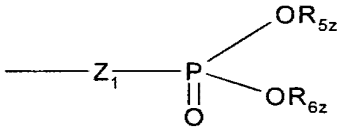
2. Use of an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined herein above, in the preparation of a medicament for preventing, inhibiting or treating PP-MS.

3. Use of claim 1 or 2, wherein the S1P receptor modulator comprises a group of formula X :



(X)

wherein Z is H, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, phenyl, phenyl substituted by OH, C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH, or CH_2-R_{4z} wherein R_{4z} is OH, acyloxy or a residue of formula (a)



(a)

wherein Z_1 is a direct bond or O, preferably O;
each of R_{5z} and R_{6z}, independently, is H, or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms;
R_{1z} is OH, acyloxy or a residue of formula (a); and each of R_{2z} and R_{3z} independently, is H, C_{1-4}alkyl or acyl.

4. Use of any preceding claim, wherein the medicament is co-administered, e.g. concomitantly or in sequence, with a VEGF-receptor antagonist, e.g. as defined hereinabove.

5. A pharmaceutical composition for use of any preceding claim, comprising an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined hereinabove, together with one or more pharmaceutically acceptable diluents or carriers therefor.

6. A pharmaceutical combination, e.g. a kit, comprising a) a first agent which is a S1P receptor modulator e.g. a compound of formulae I to XIb as defined herein above, in free form or in pharmaceutically acceptable salt form, and b) a VEGF-receptor antagonist, e.g. as defined hereinabove.

7. A method for preventing, inhibiting or treating neo-angiogenesis associated with a demyelinating disease, e.g. multiple sclerosis, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined hereinabove.

8. A method of preventing, inhibiting or treating PP-MS in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined hereinabove.

9. A method according to claim 8, wherein the S1P receptor modulator is administered intermittently.

10. A method, use, pharmaceutical composition or pharmaceutical combination of any preceding claim, wherein the S1P receptor modulator or agonist is 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, 2-amino-2-[4-(3-benzyloxyphenoxy)-2-chlorophenyl]ethyl-1,3-propane-diol, or 1-{4-[1-(4-cyclohexyl-3-trifluoromethyl-benzyloxyimino)-ethyl]-2-ethyl-benzyl}-azetidine-3-carboxylic acid, in free form or in a pharmaceutically acceptable salt form.

11. A method, use, pharmaceutical composition or pharmaceutical combination according to any one of the preceding claims, wherein the S1P receptor modulator is 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol in free form or in a pharmaceutically acceptable salt form.

Docket Number	PAT050279-US-CNT

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

UTILITY PATENT APPLICATION TRANSMITTAL AND FEE SHEET

Transmitted herewith for filing under 37 CFR § 1.53(b)(1) is a **continuation** of prior Application No. 12/303765, filed December 8, 2008.

Applicant (or identifier): Hiestand, Peter C. et al.

Title: S1P Receptor Modulators for Treating Multiple Sclerosis

Enclosed are:

1. ☒ Specification (Including Claims and Abstract) - 18 pages
2. ☐ Drawings - sheets
3. Declaration and Power of Attorney
   a. ☐ Newly executed (original or copy)
   b. ☒ Copy from a prior application (signed or with indication that original was signed)
      i. ☐ Deletion of Inventors
      Signed statement attached deleting inventor(s) named in the prior application
4. ☒ Incorporation By Reference
   The entire disclosure of the prior application, from which a copy of the Declaration and Power of Attorney is supplied under Box 3b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
5. ☐ Microfiche Computer Program (appendix)
6. Nucleotide and/or Amino Acid Sequence Submission
   ☐ Computer Readable Copy
   ☐ Paper Copy
   ☐ Statement Verifying Identity of Above Copies
7. ☒ Preliminary Amendment
8. ☐ Assignment Papers (Cover Sheet & Document(s))
9. ☐ English Translation of
10. ☒ Information Disclosure Statement
11. ☐ Certified Copy of Priority Document(s)
12. ☐ Return Receipt Postcard
13. ☒ Application Data Sheet
14. ☐ Other:

☒ The right to elect an invention or species that is different from that elected in parent Application No. 12/303765 in the event of a restriction or election of species requirement that is identical or substantially similar to that made in said parent application is hereby reserved.

Filing fee calculation:

☐ Before calculating the filing fee, please enter the enclosed Preliminary Amendment.
☐ Before calculating the filing fee, please cancel claims .

Basic Filing Fee									$ 330
Search Fee									$ 540
Examination Fee									$ 220
Multiple Dependent Claim Fee ($ 390)									$ 390
Foreign Language Surcharge ($)									$ 0
	For	Number Filed		Number Extra		Rate			
Extra Claims	Total Claims	10	-20	0	x	$ 52	=	$ 0	
	Independent Claims	3	-3	0	x	$ 220	=	$ 0	
Application Size Fee									
Total Sheets		Extra Sheets		Number of each additional 50 or fraction thereof (rounded up to a whole number)		Rate			
18	- 100	0	/50	0	x	$ 270	=	$ 0	
TOTAL FILING FEE								$ 1480	

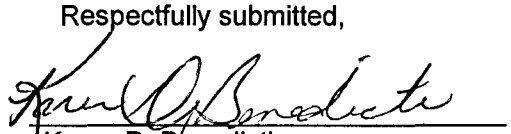
☒ Please charge Deposit Account No. 19-0134 in the name of Novartis in the amount of $1480. An additional copy of this paper is enclosed. The Commissioner is hereby authorized to charge any additional fees under 37 CFR §1.16 and §1.17 which may be required in connection with this application, or credit any overpayment, to Deposit Account No. 19-0134 in the name of Novartis.

Please address all correspondence to the address associated with Customer No. 001095, which is currently:

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936

Please direct all telephone calls to the undersigned at the number given below, and all telefaxes to (973) 781-8064.

Respectfully submitted,



Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936
(862) 778-3785

Date: 5/31/11

PTO/SB/14 (07-07)
Approved for use through 06/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	PAT050279-US-CNT
	Application Number	
Title of Invention	S1P RECEPTOR MODULATORS FOR TREATING MULTIPLE SCLEROSIS	

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.)

Applicant Information:

Applicant 1

Applicant Authority ◉ Inventor ○ Legal Representative under 35 U.S.C. 117 ○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Peter	C.	HIESTAND	

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

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Citizenship under 37 CFR 1.41(b)	AU		

Mailing Address of Applicant:

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Address 2			
City	Allschwil	State/Province	
Postal Code	4123	Country	CH

Applicant 2

Applicant Authority ◉ Inventor ○ Legal Representative under 35 U.S.C. 117 ○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Christian		SCHNELL	

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

City	Hésingue	Country Of Residence	FR
Citizenship under 37 CFR 1.41(b)	FR		

Mailing Address of Applicant:

Address 1	Rue de Buschwiller 9		
Address 2			
City	Hésingue	State/Province	
Postal Code	68220	Country	FR

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.**

PTO/SB/14 (07-07)
Approved for use through 06/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	PAT050279-US-CNT
	Application Number	
Title of Invention	S1P RECEPTOR MODULATORS FOR TREATING MULTIPLE SCLEROSIS	

Customer Number	01095		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	S1P RECEPTOR MODULATORS FOR TREATING MULTIPLE SCLEROSIS		
Attorney Docket Number	PAT050279-US-CNT	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Suggested Class (if any)		Sub Class (if any)	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)		Suggested Figure for Publication (if any)	

Publication Information:

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

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

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Enter either 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	01095		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	12/303765	2008-12-08
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12/303765	a 371 of international	PCT/EP07/05597	2007-06-25

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	PAT050279-US-CNT
	Application Number	
Title of Invention	S1P RECEPTOR MODULATORS FOR TREATING MULTIPLE SCLEROSIS	

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 benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. 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(a).

Remove

Application Number	Country	Parent Filing Date (YYYY-MM-DD)	Priority Claimed
0612721.1	GB	2006-06-27	(•) Yes ( ) No

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

Assignee Information:

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

Assignee 1

If the Assignee is an Organization check here. ☐

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

Mailing Address Information:

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

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

Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.

Signature	Karen D. Benedictis			Date (YYYY-MM-DD)	2011-05-31
First Name	Karen	Last Name	DeBenedictis	Registration Number	32977

US Case **50279-WO-PCT**

DECLARATION AND POWER OF ATTORNEY FOR UNITED STATES PATENT APPLICATION

☒Original ☐ Supplemental ☐ Substitute

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a United States patent is sought on the invention entitled

ORGANIC COMPOUNDS

the specification of which:

☐ is attached hereto.

☐ was filed on _____ (day/month/year) as Application No. _____

and, if this box (☐) contains an *

☐ was amended on _____ (day/month/year)

☒ was filed as Patent Cooperation Treaty international Application No.

PCT/EP2007/005597 on **25/June/2007** (day/month/year)

and, if this box (☐) contains an *

☐ entered the national stage in the United States and was accorded Application No.

_____

and, if this box (☐) contains an *

☐ was amended, subsequent to entry into the national stage, on _____ (day/month/year)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) specifically referred to above and, if this application was filed as a Patent Cooperation Treaty international application, by any amendments made during the international stage (including any made under Patent Cooperation Treaty Rule 91, Article 19 and Article 34).

I acknowledge my duty to disclose information which is material to patentability as defined in 37 C.F.R. 1.56, including, for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or Patent Cooperation Treaty international filing date of the continuation-in-part application.

I hereby claim the benefit under 35 U.S.C. 119(a)-(d) or (f) or 365(b) of any foreign application(s) for patent, inventor's certificate or plant breeder's right certificate listed below and under 35 U.S.C. 365(a) of any Patent Cooperation Treaty international application(s) designating at least one country other than the United States listed below and have also listed below any foreign application(s) for patent, inventor's certificate or plant breeder's right certificate and Patent Cooperation Treaty international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application the priority of which is claimed for that subject matter:

COUNTRY/REGION (OR P.C.T.)	APPLICATION No.	FILING DATE (day/month/year)	PRIORITY CLAIMED	
Great Britain	**0612721.1**	**27/June/2006**	☒ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below:

APPLICATION NO.	FILING DATE (day/month/year)

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s) listed below and under 35 U.S.C. 365(c) of any Patent Cooperation Treaty international application(s) designating the United States listed below:

United States Application No.	United States Filing Date (day/month/year)	Status (Pending, Abandoned or U.S. Patent No.)	International Application No. and Filing Date (day/month/year)

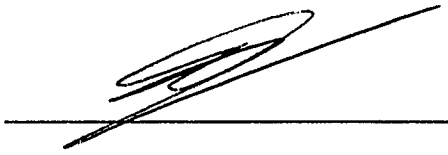
I hereby appoint all of the registered practitioners associated with Customer No. 001095, respectively and individually, as my attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

If this box (☐) contains an x ☒, I hereby authorize the registered practitioners associated with Customer No. 001095 and any others acting on my behalf to take any action relating to this application based on communications from Corporate Intellectual Property of Novartis International AG, Basle, Switzerland, or an affiliate thereof or a successor thereto, without direct communication from me.

Please send all correspondence relating to this application to the address associated with Customer No. 001095.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full name of sole or first joint inventor	**Peter C. HIESTAND**
Inventor's signature	 Date 2/07/2007 (day/month/year)
Residence	**4123 Allschwil, CH**
Citizenship	**Austria**
Post Office Address	**Schönenbuchstrasse 13a, 4123 Allschwil, CH**

Full name of second joint inventor, if any	**Christian SCHNELL**
Inventor's signature	 Date 16/7/2007 (day/month/year)
Residence	**68220 Hésingue, FR**
Citizenship	**France**
Post Office Address	**Rue de Buschwiller 9, 68220 Hésingue, FR**

IMPORTANT: Before this declaration is signed, the patent application (the specification, the claims and this declaration) must be read and understood by each person signing it, and no changes may be made in the application after this declaration has been signed.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO: Not Yet Known
FILED: Herewith
FOR: S1P Receptor Modulators for Treating Multiple Sclerosis

Art Unit:
Examiner:

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Sir:

Prior to the examination of the above-referenced patent application, please enter the following preliminary amendments.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of the claims which begins on page 4 of this paper.

Remarks/Arguments begin on page 6 of this paper.

Amendments to the Specification:

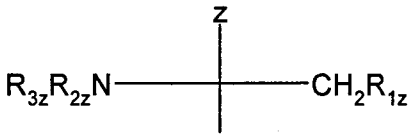
Please insert the following as the first paragraph beneath the title on page 1:

This application is a Continuation of U.S. Application No. 12/303765 filed December 8, 2008 which is a 371 of PCT/EP2007/005597 filed on June 25, 2007, which claims benefit of Great Britain Application No. 0612721.1 filed on June 27, 2006, which in their entirety are herein incorporated by reference.—

A copy of the abstract is herein provided on the following separate sheet.

Abstract

The present invention relates uses of an S1P receptor modulator such as 2-substituted 2-amino-propane-1,3-diol or 2-amino-propanol derivatives, e. g. a compound comprising a group of formula X



(X)

for the treatment or prevention of neo-angiogenesis associated with a demyelinating disease, e.g. multiple sclerosis.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1 – 11. (Cancelled).

Claim 12. (New) A method for inhibiting or treating neo-angiogenesis associated with multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 13. (New) A method for reducing or alleviating relapses in multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 14. (New) A method according to claim 12 for inhibiting or treating neo-angiogenesis associated with relapsing-remitting multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 15. (New) A method according to claim 13 for reducing or alleviating relapses in relapsing-remitting multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 16. (New) A method according to claim 12 or 13 wherein 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride is administered.

Claim 17. (New) A method for slowing progression of multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 18. (New) A method according to claim 17 wherein the disease is relapsing-remitting multiple sclerosis.

Claim 19. (New) A method according to claim 17 wherein the subject is in a relapsing-remitting phase of the disease.

Claim 20. (New) A method according to claim 17 wherein the disease is primary-progressive multiple sclerosis (PP-MS).

Claim 21. (New) A method according to claim 17, wherein 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride is administered.

REMARKS/ARGUMENTS

The foregoing amendments to the specification are to insert the cross-reference beneath the title and to place the Abstract on a separate sheet. The amendments to the claims are to place the claims in better form and remove multiple dependencies. No new matter has been added. Should the Examiner have any questions, please contact the undersigned attorney.

Respectfully submitted,

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Date: 5/31/11

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO:
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Examiner:

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INFORMATION DISCLOSURE STATEMENT

Sir:

This paper is being filed:

☒ within three months of the filing date of the application. Therefore, no fees are required.

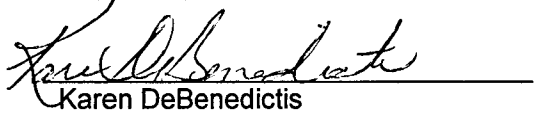
If a fee is deemed to be required, the Commissioner is hereby authorized to charge such fee to Deposit Account No. 19-0134 in the name of Novartis.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO/SB/08A/B.

☒ The references are of record in parent Application No. 12/303765 filed December 8, 2008, and copies are available therein.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO/SB/08A/B form(s).

Respectfully submitted,



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