8359292

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

March 28, 2023

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

APPLICATION NUMBER: *15/260,888*
FILING DATE: *September 9, 2016*
PATENT NUMBER: *9,725,504*
ISSUE DATE: *August 8, 2017*

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

M. Tarver

Miguel Tarver
Certifying Officer

ALXN2103
IPR2023-00933

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 15260888 Document Date: 09/09/2016

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

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- Sequence Listing

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Form Revision Date: August 26, 2013

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: September 9, 2016
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: Not Yet Assigned

Confirmation No.: N/A

Filed: Concurrently Herewith

Art Unit: N/A

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: Not Yet Assigned

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

FIRST PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

Dear Sir:

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

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

Remarks/Arguments begin on page 3 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please insert the following paragraphs after the title:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Patent Application No. 15/148,839, filed May 6, 2016, which is a continuation of U.S. Patent Application No. 13/426,973, filed March 22, 2012, which is a continuation of U.S. Patent Application No. 12/225,040, filed on May 13, 2009, which is a 35 U.S.C. 371 national stage filing of International Application No. PCT/US2007/006606, filed March 15, 2007. The contents of the aforementioned applications are hereby incorporated by reference in their entireties.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 8, 2016, is named AXJ_114BUSCN3_Seq_Listing.txt and is 10,483 bytes in size.

Samsung Bioepis Exhibit No. 1002
Page 004

REMARKS

Amendments to the Specification

Applicant has amended the specification at page 1 to update the related applications and Sequence Listing sections. *No new matter has been added.*

CONCLUSION

If a telephone conversation with Applicant's Attorney would expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 217-4630.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3.

Dated: September 9, 2016

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY &
SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630 (617) 742-4214 (Fax)
Attorney/Agent For Applicant

Samsung Bioepis Exhibit No. 1002
Page 005

Electronic Patent Application Fee Transmittal	
Application Number:	
Filing Date:	
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Attorney Docket Number:	AXJ-114BUSCN3
Filed as Large Entity	
Filing Fees for Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	280	280
Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Request for Prioritized Examination	1817	1	4000	4000
Pages:				
Claims:				
Miscellaneous-Filing:				
PROCESSING FEE, EXCEPT PROV. APPLS.	1830	1	140	140

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**5740**

Electronic Acknowledgement Receipt	
EFS ID:	26877080
Application Number:	15260888
International Application Number:	
Confirmation Number:	2558
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3
Receipt Date:	09-SEP-2016
Filing Date:	
Time Stamp:	14:43:55
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$5740
RAM confirmation Number	1442
Deposit Account	120080
Authorized User	SLOPER, JILL G.
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 CFR 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 CFR 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 CFR 1.19 (Document supply fees)

Charge any Additional Fees required under 37 CFR 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 CFR 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	Application Data Sheet	AXJ_114BUSCN3_ADS.pdf	62210 2916f90aa33954f60b30947edfad8ea13b903717	no	9
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
2	Drawings-only black and white line drawings	AXJ_114BUSCN3_Figures.pdf	990652 d39f7fb9a45e3f53a1be19b0561ddf1307db5f45	no	4
Warnings:					
Information:					
3	Oath or Declaration filed	AXJ_114BUSCN3_Dec.pdf	2443083 aec88d61e9a45b53a97b118d2d8ea79a0fe39d07	no	3
Warnings:					
Information:					
4	Power of Attorney	AXJ_114BUSCN3_POA.pdf	210805 eaf70c0c70a173443ced1184ec018edf9d87b414	no	2
Warnings:					
Information:					
5	CRF Statement Paper and CRF are the same	AXJ_114BUSCN3_Statement_re_SL.pdf	110908 e28e603ef56e1921be272a74dfd10b2cb7ba9f9f	no	2
Warnings:					
Information:					
6	TrackOne Request	AXJ_114BUSCN3_TrackOneReq.pdf	109652 1d2fb0b348de3c9308aeb802a89e50d34d181545	no	1
Warnings:					

Information:					
7		AXJ_114BUSCN3_Specification.pdf	4029306 c54764b0bad6f0be2b38bf47d9a2020365ea2503	yes	47
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Specification		1	45	
	Claims		46	46	
	Abstract		47	47	
Warnings:					
Information:					
8	Sequence Listing (Text File)	AXJ_114BUSCN3_Seq_Listing.txt	10483	no	-
Warnings:					
Information:					
9		AXJ_114BUSCN3_PrelimAmdt.pdf	135323 82e536fdb5dc6640f139c3b76111521ca2884073	yes	3
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Preliminary Amendment		1	1	
	Specification		2	2	
	Applicant Arguments/Remarks Made in an Amendment		3	3	
Warnings:					
Information:					
10	Fee Worksheet (SB06)	fee-info.pdf	38607 5c46badbaf199fae985904b6fbac66821317eee6	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	8141029		

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

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

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

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

PTO/AIA/14 (11-15)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AXJ-114BUSCN3
	Application Number	
Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT	

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

Secrecy Order 37 CFR 5.2:

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

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Leonard		BELL	

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

City	Woodbridge	State/Province	CT	Country of Residence	US

Mailing Address of Inventor:

Address 1	59 Tumblebrook Road		
Address 2			
City	Woodbridge	State/Province	CT
Postal Code	06525	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Russell	P.	ROTHER	

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

City	Oklahoma City	State/Province	OK	Country of Residence	US

Mailing Address of Inventor:

Address 1	10325 Forest Meadow Drive		
Address 2			
City	Oklahoma City	State/Province	OK
Postal Code	73151	Country	US

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

Correspondence Information:

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

PTO/AIA/14 (11-15)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AXJ-114BUSCN3
	Application Number	
Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT	

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

Customer Number	133485		
Email Address	IPBoston.Docketing@nelsonmullins.com	Add Email	Remove Email

Application Information:

Title of the Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT		
Attorney Docket Number	AXJ-114BUSCN3	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	4	**Suggested Figure for Publication (if any)**	

Filing By Reference:

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

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

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

Publication Information:

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

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

Representative Information:

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

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	133485		

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AXJ-114BUSCN3
	Application Number	
Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT	

Domestic Benefit/National Stage Information:

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

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	15/148839	2016-05-06
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
15/148839	Continuation of	13/426973	2012-03-22
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
13/426973	Continuation of	12/225040	2009-05-13
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
12/225040	a 371 of international	PCT/US2007/006606	2007-03-15

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

Foreign Priority Information:

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

Remove

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

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

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AXJ-114BUSCN3
	Application Number	
Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT	

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

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

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AXJ-114BUSCN3
	Application Number	
Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT	

Authorization or Opt-Out of Authorization to Permit Access:

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

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

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

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

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

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

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

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

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

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

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

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AXJ-114BUSCN3
	Application Number	
Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT	

Applicant Information:

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

Applicant 1

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

Clear

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

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

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	Alexion Pharmaceuticals, Inc.

Mailing Address Information For Applicant:

Address 1	100 College St.		
Address 2			
City	New Haven	**State/Province**	CT
Country	US	Postal Code	06510
Phone Number		Fax Number	
Email Address			

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

Assignee Information including Non-Applicant Assignee Information:

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

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	AXJ-114BUSCN3
	Application Number	
Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT	

Assignee 1

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

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

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country		Postal Code	
Phone Number		Fax Number	
Email Address			

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

Signature:

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

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

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

Signature	/Jill Gorny Sloper, Esq./			Date (YYYY-MM-DD)	2016-09-09
First Name	Jill Gorny	Last Name	Sloper	Registration Number	60760

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

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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	Application Number	
Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT	

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

Privacy Act Statement

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

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

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

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

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

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

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

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

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

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

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

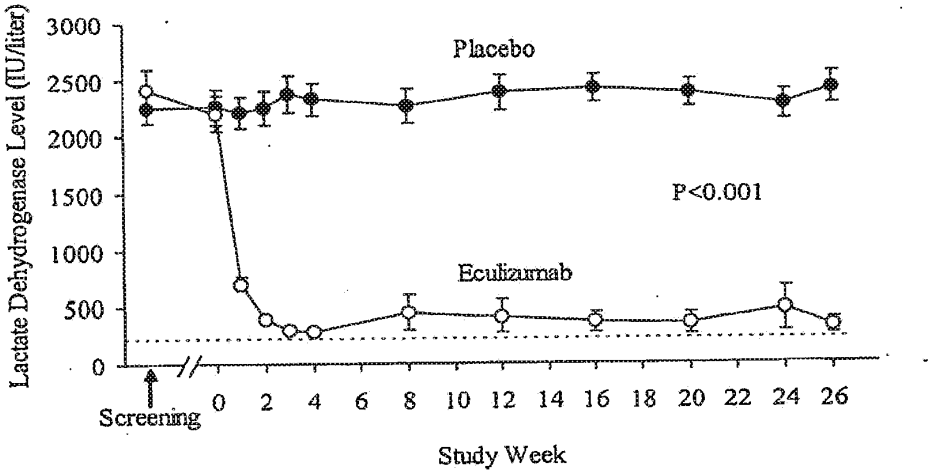
Lactate Dehydrogenase Level (IU/liter)
3000
2500
2000
1500
1000
500
0
Placebo
P<0.001
Eculizumab
Screening
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Study Week

FIGURE 1A

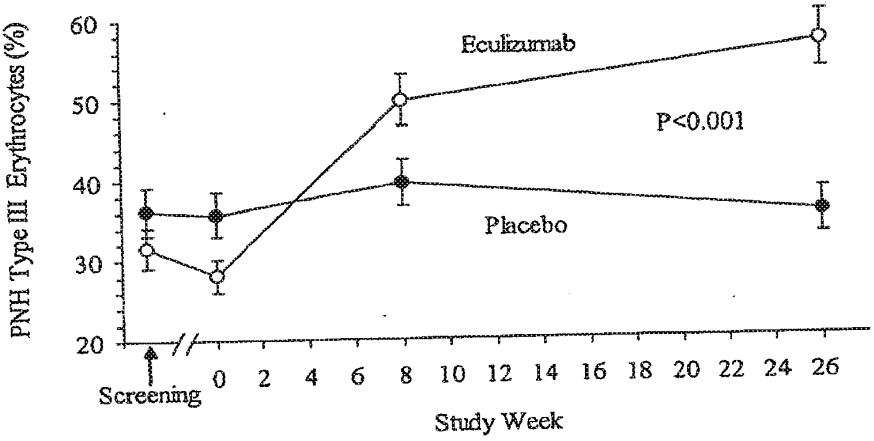
PNH Type III Erythrocytes (%)
60
50
40
30
20
Eculizumab
P<0.001
Placebo
Screening
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Study Week

FIGURE 1B

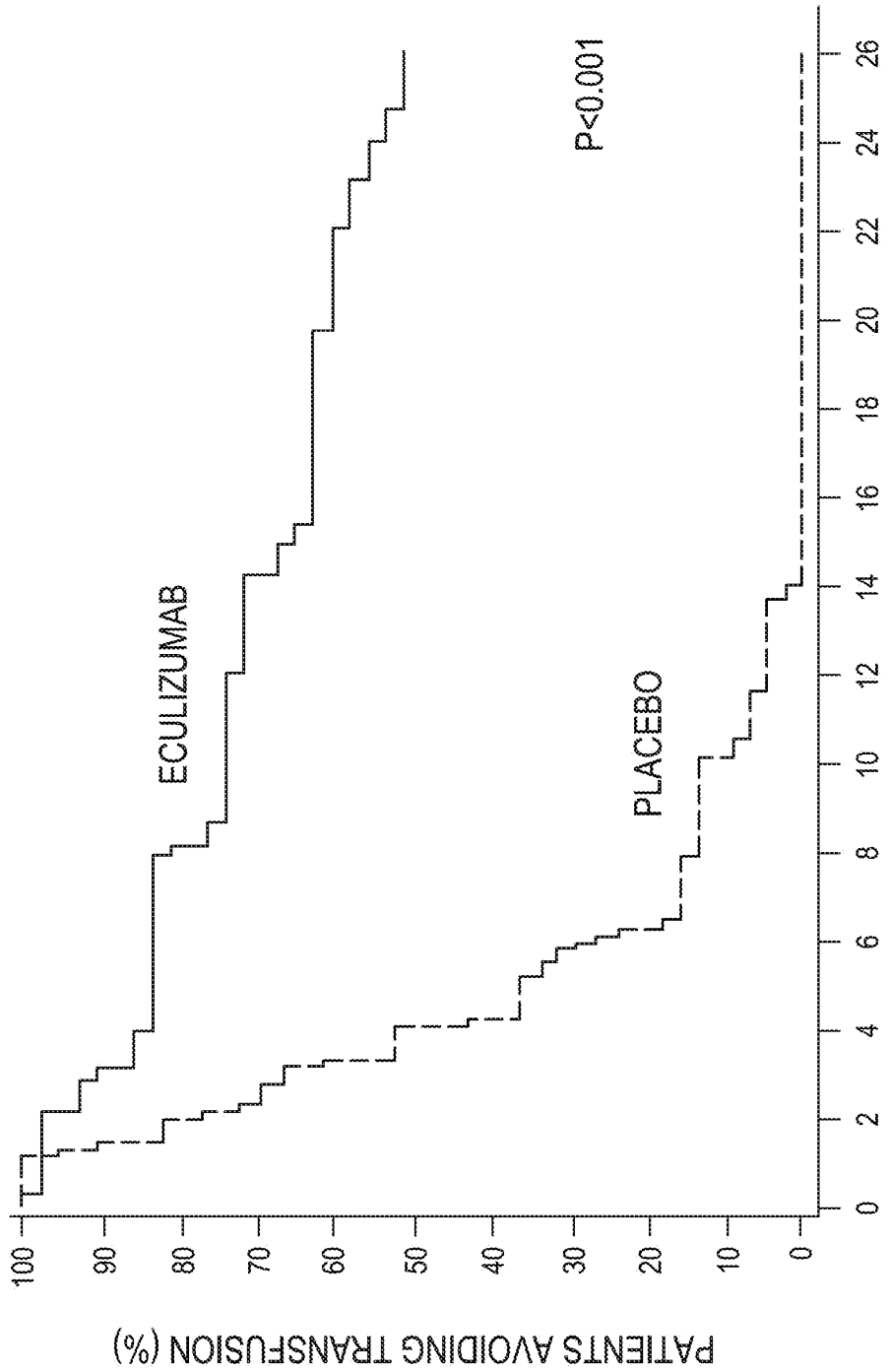
PATIENTS AVOIDING TRANSFUSION (%)
100
90
80
70
60
50
40
30
20
10
0
ECULIZUMAB
PLACEBO
P<0.001
0
2
4
6
8
10
12
14
16
18
20
22
24
26
STUDY WEEK

FIGURE 2

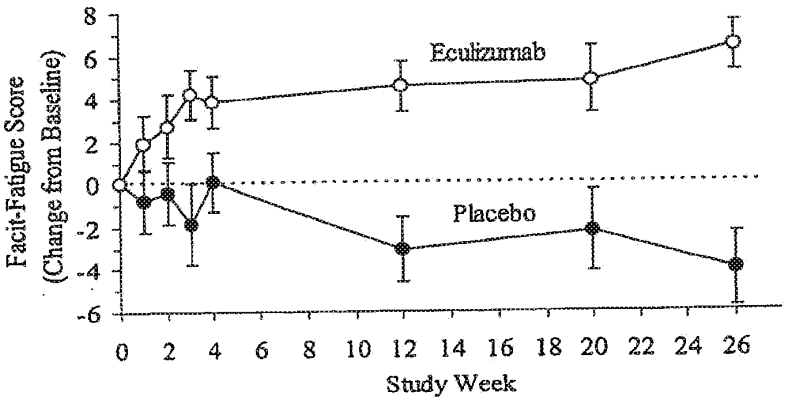
Facit-Fatigue Score
(Change from Baseline)
8
6
4
2
0
-2
-4
-6
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Study Week
Eculizumab
Placebo

FIGURE 3

PTO/AIA/01 (06-12)
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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

As the below named inventor, I hereby declare that:

This declaration is directed to:
- [ ] The attached application, or
- [x] United States application or PCT international application number 15/148,839 filed on 05/06/2016

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

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

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

WARNING:

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

LEGAL NAME OF INVENTOR

Inventor: Leonard Bell Date (Optional): _____

Signature: _____

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

Doc code: Oath
Document Description: Oath or declaration filed

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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

This statement is directed to:

[ ] The attached application,

OR

[X] United States application or PCT international application number 15/148,839 filed on 05/06/2016.

LEGAL NAME of inventor to whom this substitute statement applies:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

Russell P. ROTHER

Residence (except for a deceased or legally incapacitated inventor):

Oklahoma City	OK	United States of America
City	State	Country

Mailing Address (except for a deceased or legally incapacitated inventor):

10325 Forest Meadow Drive

Oklahoma City	OK	73151	United States of America
City	State	Zip	Country

I believe the above-named inventor or joint inventor to be the original inventor or an original joint inventor of a claimed invention in the application.

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

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

Relationship to the inventor to whom this substitute statement applies:

[ ] Legal Representative (for deceased or legally incapacitated inventor only),

[X] Assignee,

[ ] Person to whom the inventor is under an obligation to assign,

[ ] Person who otherwise shows a sufficient proprietary interest in the matter (petition under 37 CFR 1.46 is required), or

[ ] Joint Inventor.

PTO/SB/AIA02 (07-13)
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SUBSTITUTE STATEMENT

Circumstances permitting execution of this substitute statement:

- [ ] Inventor is deceased,
- [ ] Inventor is under legal incapacity,
- [ ] Inventor cannot be found or reached after diligent effort, or
- [x] Inventor has refused to execute the oath or declaration under 37 CFR 1.63.

If there are joint inventors, please check the appropriate box below:

- [x] An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) naming the entire inventive entity has been or is currently submitted.

OR

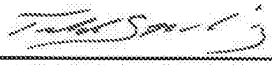
- [ ] An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) has not been submitted. Thus, a Substitute Statement Supplemental Sheet (PTO/AIA/11 or equivalent) naming the entire inventive entity and providing inventor information is attached. See 37 CFR 1.64(b).

WARNING:

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

PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

Name: Todd Spalding	Date (Optional):

Signature: 

APPLICANT NAME AND TITLE OF PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

If the applicant is a juristic entity, list the applicant name and the title of the signer:

Applicant Name: Alexion Pharmaceuticals, Inc.

Title of Person Executing This Substitute Statement: Vice President, Head of Intellectual Property

The signer, whose title is supplied above, is authorized to act on behalf of the applicant.

Residence of the signer (unless provided in an application data sheet, PTO/AIA/14 or equivalent):

City	State	Country

Mailing Address of the signer (unless provided in an application data sheet, PTO/AIA/14 or equivalent)

100 College St.

City	State	Zip	Country
New Haven	CT	06510	US

Note: Use an additional PTO/AIA/02 form for each inventor who is deceased, legally incapacitated, cannot be found or reached after diligent effort, or has refused to execute the oath or declaration under 37 CFR 1.63.

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82A (07-13)
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TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.

Application Number	Not Yet Assigned
Filing Date	Concurrently Herewith
First Named Inventor	Leonard BELL
Title	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	AXJ-114BUSCN3

SIGNATURE of Applicant or Patent Practitioner

Signature	/Jill Gorny Sloper, Esq./	Date (Optional)	September 9, 2016
Name	Jill Gorny Sloper, Esq.	Registration Number	60,760
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)			

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

☐ *Total of 1 forms are submitted.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: September 9, 2016 Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
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POWER OF ATTORNEY BY APPLICANT

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

Application Number	Filing Date

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

[X] I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 133485

OR

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

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

[X] The address associated with the above-mentioned Customer Number

OR

[ ] The address associated with Customer Number:

OR

Firm or Individual Name	Jane E. Remillard, Esq. NELSON MULLINS RILEY & SCARBOROUGH LLP				
Address	One Post Office Square				
City	Boston	State	MA	Zip	02109-2127
Country	US				
Telephone	(800) 237-2000	Email	IPBoston.Docketing@nelsonmullins.com		

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

Alexion Pharmaceuticals, Inc.

[ ] Inventor or Joint Inventor (title not required below)

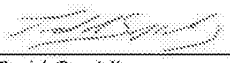
[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

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

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

SIGNATURE of Applicant for Patent

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

Signature		Date (Optional)	07/20/15
Name	Todd Spalding		
Title	Vice President, Head of Intellectual Property		

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

[ ] Total of 1 forms are submitted.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: September 9, 2016
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: Not Yet Assigned

Confirmation No.: N/A

Filed: Concurrently Herewith

Art Unit: N/A

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: Not Yet Assigned

MS Sequence
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT PURSUANT TO 37 CFR 1.821

Dear Sir:

Submitted herewith in connection with the above-referenced patent application and in full compliance with 37 C.F.R. §§1.821-1.825 is a computer readable copy and paper copy of the Sequence Listing (filed together as a .txt file via the United States Patent Office's Electronic Filing System).

I hereby state that I have reviewed the paper copy of the Sequence Listing, as required by 37 CFR 1.821(c), and have reviewed the computer readable form of the Sequence Listing, as required by 37 CFR 1.821(e), and that the content of the paper and computer readable copies for the above-referenced Patent application are the same as required by 37 CFR 1.821(f) (note that these documents are submitted as one electronic file). No new matter has been added to the Sequence Listing.

Dated: September 9, 2016

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY &
SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630 (617) 742-4214 (Fax)
Attorney/Agent For Applicant

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (04-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Leonard BELL	Nonprovisional Application Number (if known):	Not Yet Assigned
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently $0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.
2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.
3. The applicable box is checked below:

I. [x] **Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)**

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. [ ] **Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)**

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature	/Jill Gorny Sloper, Esq./	Date	September 9, 2016
Name (Print/Typed)	Jill Gorny Sloper, Esq.	Practitioner Registration Number	60,760

***Note**: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

[ ] *Total of 1 forms are submitted.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: September 9, 2016 Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

BACKGROUND

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematologic disease that results from clonal expansion of hematopoietic stem cells with somatic mutations in the X-linked gene called PIG-A.[1,2] Mutations in PIG-A lead to an early block in the synthesis of glycosylphosphatidylinositol (GPI)-anchors, which are required to tether many proteins to the cell surface. Consequently, PNH blood cells have a partial (type II) or complete (type III) deficiency of GPI-anchored proteins.

Intravascular hemolysis is a prominent feature of PNH and a direct result of the absence of the GPI-anchored complement regulatory protein CD59.[3,4] Under normal circumstances, CD59 blocks the formation of the terminal complement complex (also called the membrane attack complex) on the cell surface, thereby preventing erythrocyte lysis and platelet activation.[5-8] Excessive or persistent intravascular hemolysis in PNH patients not only results in anemia (normal ranges of hemoglobin are 14-18 g/dL for men and 12-16 g/dL for women, and persons with lower levels are considered to be anemic), but also hemoglobinuria and clinical sequelae related to the release of the erythrocyte contents into the circulation: fatigue, thrombosis, abdominal pain, dysphagia, erectile dysfunction, and pulmonary hypertension.[9,10,21,22] Indeed, impaired quality of life in PNH is disproportionate to the degree of anemia. Many PNH patients depend on blood transfusions to maintain adequate erythrocyte hemoglobin levels. There have been no therapies that effectively reduce intravascular hemolysis and improve the associated clinical morbidities in PNH.

Eculizumab is a humanized monoclonal antibody directed against the terminal complement protein C5.[11] In a preliminary, 12-week, open-label clinical study in 11 PNH patients, eculizumab was shown to reduce intravascular hemolysis and transfusion requirements.[12] However, this unblinded study involved a small number of patients with no control arm and without protocol-driven transfusion standards.

SUMMARY

The present pivotal, phase III study, Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria (TRIUMPH), evaluated the effect of eculizumab on the stabilization of hemoglobin levels and transfusion requirements during 6 months of treatment in a cohort of 87 transfusion-dependent PNH patients. Measures of intravascular hemolysis and quality of life were also assessed. This is the first placebo controlled study of a PNH patient population to control hemolysis and to differentiate between the effects due to hemolysis and the effects due to anemia.

It has been surprisingly discovered that certain aspects of quality of life were unexpectedly improved by the treatment of PNH patients with eculizumab. Furthermore, these improvements in the quality of life were independent of transfusion. The improved aspects include, e.g., global health status, physical functioning, emotional functioning, cognitive functioning, role functioning, social functioning, fatigue, pain, dyspnea, appetite loss and insomnia. Improvement was also seen in nausea and vomiting, diarrhea, constipation, and financial difficulties but did not reach the level of statistical significance. Because the treated patients remained anemic throughout their treatment, it was unexpected that all of these improvements would have been seen because they were previously thought to be a result of the patient being anemic. Although not wishing to be bound by any theory, it appears that some of the symptoms are likely due, at least in part, to hemolysis and release of hemoglobin into the bloodstream and do not result solely from the patient being anemic. The treatment with eculizumab decreases the amount of lysis thereby limiting hemoglobin release into the bloodstream, thereby apparently resulting in the improvements seen in the treated patients' quality of life. The results presented herein indicate that any treatment that decreases hemolysis in a patient will result in an improvement in the quality of life of said patient.

In certain aspects, the application provides a method to improve at least one aspect of the quality of life of a patient suffering from paroxysmal nocturnal hemoglobinuria, said method comprising administering to said patient in need thereof a compound which inhibits complement or inhibits formation of C5b-9.

In certain aspects, the application provides a method to improve at least one aspect of the quality of life of a patient suffering from paroxysmal nocturnal hemoglobinuria, said method comprising administering to said patient in need thereof a compound which inhibits

intravascular hemolysis. In certain embodiments, said method results in a greater than 30% reduction in LDH in said patient.

In certain aspects, the application provides a method to improve at least one aspect of the quality of life of an anemic patient whose anemia results at least in part from hemolysis, said method comprising administering to said patient in need thereof a compound which inhibits intravascular hemolysis, wherein said patient remains anemic. In certain embodiments, said method results in a greater than 30% reduction in LDH in said patient.

In certain aspects, the application provides a method of prolonging the health-adjusted life expectancy of a patient comprising administering to said patient in need thereof a compound which inhibits formation of C5b-9. In certain embodiments, said patient is anemic. In certain embodiments, said patient remains anemic following treatment. In certain embodiments, said patient has a hemoglobin level less than i) 14 g/dL if a man or ii) 12 g/dL if a woman. In certain embodiments, said patient has a hemoglobin level less than i) 13 g/dL if a man or ii) 11 g/dL if a woman. In certain embodiments, said patient has a hemoglobin level less than i) 12 g/dL if a man or ii) 10 g/dL if a woman. In certain embodiments, said patient suffers from paroxysmal nocturnal hemoglobinuria.

In certain aspects, the application provides a pharmaceutical composition comprising an antibody that binds C5 or an active antibody fragment thereof. In certain embodiments, the antibody that binds C5 or an active antibody fragment thereof is eculizumab. In certain embodiments, the antibody that binds C5 or an active antibody fragment thereof is pexelizumab. In certain embodiments, the pharmaceutical formulations of the application may be administered to a subject, particularly a subject having PNH.

In certain aspects, the application provides a method of treating a patient suffering from paroxysmal nocturnal hemoglobinuria by administering a pharmaceutical composition comprising an antibody that binds C5 or an active antibody fragment thereof. In certain embodiments, the antibody that binds C5 or an active antibody fragment thereof is eculizumab. In certain embodiments, the antibody that binds C5 or an active antibody fragment thereof is pexelizumab. In certain embodiments, the pharmaceutical formulations of the application may be administered to a subject, particularly a subject having PNH.

In certain aspects, the application provides kits comprising a pharmaceutical composition of the application. In some embodiments, the kit further comprises at least one component of a closed sterile system. Components of the closed sterile system

include, but are not limited to, needles, syringes, catheter based syringes, needle based injection devices, needle-less injection devices, filters, tubing, valves and cannulas. In a related embodiment, the kit comprise components for the removal of a preservative from the composition. Such components include filters, syringes, vials, containers, tubing, etc.

In certain embodiments, said quality of life is measured by a FACIT-Fatigue score. In certain embodiments, the FACIT-Fatigue score increases by at least 3 points. In certain embodiments, the FACIT-Fatigue score increases by ≥ 4 points.

In certain embodiments, said quality of life is measured by an EORTC QLQ-C30 score. In certain embodiments, said EORTC QLQ-C30 score improves by $\geq 10\%$ of the pretreatment score. In certain embodiments, said aspect of the quality of life as measured by an EORTC QLQ-C30 score is selected from the group consisting of a) global health status, b) physical functioning, c) emotional functioning, d) cognitive functioning, e) role functioning, f) social functioning, g) fatigue, h) pain, i) dyspnea, j) appetite loss, and k) insomnia. In certain embodiments, said aspect of quality of life is fatigue.

In certain embodiments, said compound is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH. In certain embodiments, said compound is a steroid that suppresses complement.

In certain embodiments, said compound is selected from the group consisting of antibodies, active antibody fragments, soluble complement inhibitory compounds, proteins, soluble complement inhibitors with a lipid tail, protein fragments, peptides, small organic compounds, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors. In certain embodiments, said compound is an antibody or an active antibody fragment. In certain embodiments, said antibody or active antibody fragment is selected from the group consisting of a) polyclonal antibodies, b) monoclonal antibodies, c) single chain antibodies, d) chimeric antibodies, e) humanized antibodies, f) Fabs, g) F(ab')s, h) $F(ab')_2$s, i) Fvs, j) diabodies, and k) human antibodies.

In certain embodiments, said antibody or an active antibody fragment thereof binds C5. In certain embodiments, said antibody or active antibody fragment blocks C5 cleavage. In certain embodiments, said antibody or active antibody fragment inhibits the formation of C5b-9. In certain embodiments, said antibody is eculizumab. In certain embodiments, said antibody or active antibody fragment is administered for at least 6

months. In certain embodiments, said patient has aplastic anemia or myelodysplastic syndrome.

In certain embodiments, said antibody that binds C5 or an active antibody fragment thereof is administered in a single unit dosage form. In certain embodiments, the single unit dosage form is a 300 mg unit dosage form. In certain embodiments, the single unit dosage form is lyophilized. In certain embodiments, the single unit dosage form is a sterile solution. In certain embodiments, the single unit dosage form is a preservative free formulation. In certain embodiments, the 300 mg single-use dosage form comprises 30 ml of a 10 mg/ml sterile, preservative free solution.

In certain embodiments, the antibody that binds C5 or an active antibody fragment thereof comprises an altered constant region, wherein said antibody or antigen-binding fragment exhibits decreased effector function relative to an anti-CDCP1 antibody with a native constant region. In certain embodiments, decreased effector function comprises one or more properties of the following group: a) decreased antibody-dependent cell-mediated cytotoxicity (ADCC), and b) decreased complement dependent cytotoxicity (CDC), compared to an anti-CDCP1 antibody with a native constant region. In certain embodiments, the altered constant region comprises a G2/G4 construct in place of the G1 domain.

In certain embodiments, the antibody that binds C5 or an active antibody fragment thereof comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises one or more CDR regions having an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7, and wherein the light chain variable region comprises one or more CDR regions having an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10. In certain embodiments, the antibody that binds C5 or an active antibody fragment thereof comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region consists of SEQ ID NO: 1 and the light chain variable region consists of SEQ ID NO: 3. In certain embodiments, the pharmaceutical composition comprises eculizumab. In certain embodiments, the pharmaceutical composition comprises pexelizumab. In certain embodiments, the antibody that binds C5 or an active antibody fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain consists of SEQ ID NO: 2 and the light chain consists of SEQ ID NO: 4.

In certain embodiments, said patient is anemic. In certain embodiments, said patient remains anemic following treatment. In certain embodiments, said patient has a hemoglobin level less than i) 14 g/dL if a man or ii) 12 g/dL if a woman. In certain embodiments, said patient has a hemoglobin level less than i) 13 g/dL if a man or ii) 11 g/dL if a woman. In certain embodiments, said patient has a hemoglobin level less than i) 12 g/dL if a man or ii) 10 g/dL if a woman.

In certain embodiments, said health-adjusted life expectancy is measured according to a unit selected from the group consisting of Years of potential life lost, Disability-free life expectancy, Health-adjusted life year, Quality adjusted life year, Healthy years equivalents, Healthy days gained, Episode-free day, Q-TWiST, Health Utilities Index, or Years of healthy life.

In certain embodiments, the health-adjusted life expectancy in a subject is prolonged by at least one day. In certain embodiments, the health-adjusted life expectancy in a subject is prolonged by at least week. In certain embodiments, the health-adjusted life expectancy in a subject is prolonged by at least one month. In certain embodiments, the health-adjusted life expectancy in a subject is prolonged by at least one year.

In certain embodiments, the pharmaceutical composition is in a single unit dosage form. In certain embodiments, the single unit dosage form is a 300 mg unit dosage form. In certain embodiments, the pharmaceutical composition is lyophilized. In certain embodiments, the pharmaceutical composition is a sterile solution. In certain embodiments, the pharmaceutical composition is a preservative free formulation. In certain embodiments, the pharmaceutical composition comprises a 300 mg single-use formulation of 30 ml of a 10 mg/ml sterile, preservative free solution. In certain embodiments, the pharmaceutical composition comprises an antibody that binds C5 or an active antibody fragment thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-B show that eculizumab treatment decreases intravasoular hemolysis and increases PNH type III erythrocytes. Figure 1A shows the degree of intravascular hemolysis in PNH patients, demonstrated by mean lactate dehydrogenase (LDH) levels. Figure 1B shows the mean proportion of PNH type III erythrocytes assessed for placebo- and eculizumab-treated patients.

Figure 2 shows the effect of eculizumab treatment on transfusion requirements in PNH patients. This is a Kaplan-Meier plot of time to first transfusion for eculizumab- and placebo-treated patients from baseline through week 26. The P value is from the log rank analysis.

Figure 3 shows the effect of eculizumab on fatigue assessed by the FACIT-Fatigue Instrument. Quality of Life scores were assessed using the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) instrument. Values for change from baseline to 26 weeks represent least-square means. A positive change indicates an improvement and a negative change indicates deterioration in the FACIT-Fatigue measures of quality of life.

DETAILED DESCRIPTION

I. Definitions

The term "derived from" means "obtained from" or "produced by" or "descending from".

The term "genetically altered antibodies" means antibodies wherein the amino acid sequence has been varied from that of a native antibody. Because of the relevance of recombinant DNA techniques to this application, one need not be confined to the sequences of amino acids found in natural antibodies; antibodies can be redesigned to obtain desired characteristics. The possible variations are many and range from the changing of just one or a few amino acids to the complete redesign of, for example, the variable or constant region. Changes in the constant region will, in general, be made in order to improve or alter characteristics, such as complement fixation, interaction with membranes and other effector functions. Changes in the variable region will be made in order to improve the antigen binding characteristics.

The term "an antigen-binding fragment of an antibody" refers to any portion of an antibody that retains the binding utility to the antigen. An exemplary antigen-binding fragment of an antibody is the heavy chain and/or light chain CDR, or the heavy and/or light chain variable region.

The term "homologous," in the context of two nucleic acids or polypeptides refers to two or more sequences or subsequences that have at least about 85%, at least 90%, at least 95%, or higher nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using the following sequence comparison

method and/or by visual inspection. In certain embodiments, the "homolog" exists over a region of the sequences that is about 50 residues in length, at least about 100 residues, at least about 150 residues, or over the full length of the two sequences to be compared.

Methods of determining percent identity are known in the art. "Percent (%) sequence identity" with respect to a specified subject sequence, or a specified portion thereof, may be defined as the percentage of nucleotides or amino acids in the candidate derivative sequence identical with the nucleotides or amino acids in the subject sequence (or specified portion thereof), after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent sequence identity, as generated by the program WU-BLAST-2.0a19 (Altschul et al., J. Mol. Biol. 215:403-410 (1997); http://blast.wustl.edu/blast/README.html) with search parameters set to default values. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched. A "% identity value" is determined by the number of matching identical nucleotides or amino acids divided by the sequence length for which the percent identity is being reported.

II. Overview

The present disclosure relates to a method of treating paroxysmal nocturnal hemoglobinuria ("PNH"), more specifically to improving certain aspects of quality of life which are impaired in PNH patients, and other hemolytic diseases in mammals. Specifically, the methods of treating hemolytic diseases, which are described herein, involve using compounds which bind to or otherwise block the generation and/or activity of one or more complement components. The present methods have been found to provide surprising results. For instance, hemolysis rapidly ceases upon administration of the compound which binds to or otherwise blocks the generation and/or activity of one or more complement components, with hemoglobinuria being significantly reduced after treatment. Also, hemolytic patients can be rendered less dependent on transfusions or transfusion-independent for extended periods (twelve months or more), well beyond the 120 day life cycle of red blood cells. In addition, type III red blood cell count can be increased dramatically in the midst of other mechanisms of red blood cell lysis (non-complement mediated and/or earlier complement component mediated e.g., Ch3). Another example of a surprising result is that symptoms resolved, indicating that NO serum levels were increased

enough even in the presence of other mechanisms of red blood cell lysis. These and other results reported herein are unexpected and could not be predicted from prior treatments of hemolytic diseases.

III The Complement System

The complement system, useful complement inhibitors, and use of these inhibitors to treat PNH and other patients are more fully described in PCT Patent Application PCT/US2005/003225 filed February 3, 2005 and published as International Publication Number WO 2005/074607 A2 on August 18, 2005, the contents of which are incorporated herein by reference in their entirety.

The complement system acts in conjunction with other immunological systems of the body to defend against intrusion of cellular and viral pathogens. There are at least 25 complement proteins, which are found as a complex collection of plasma proteins and membrane cofactors. The plasma proteins make up about 10% of the globulins in vertebrate serum. Complement components achieve their immune defensive functions by interacting in a series of intricate but precise enzymatic cleavage and membrane binding events. The resulting complement cascade leads to the production of products with opsonic, immunoregulatory, and lytic functions.

The complement cascade progresses via the classical pathway or the alternative pathway. These pathways share many components and, while they differ in their initial steps, they converge and share the same "terminal complement" components (C5 through C9) responsible for the activation and destruction of target cells.

The classical complement pathway is typically initiated by antibody recognition of and binding to an antigenic site on a target cell. The alternative pathway is usually antibody independent and can be initiated by certain molecules on pathogen surfaces. Both pathways converge at the point where complement component C3 is cleaved by an active protease (which is different in each pathway) to yield C3a and C3b. Other pathways activating complement attack can act later in the sequence of events leading to various aspects of complement function.

C3a is an anaphylatoxin. C3b binds to bacterial and other cells, as well as to certain viruses and immune complexes, and tags them for removal from the circulation. C3b in this role is known as opsonin. The opsonic function of C3b is considered to be the most

important anti-infective action of the complement system. Patients with genetic lesions that block C3b function are prone to infection by a broad variety of pathogenic organisms, while patients with lesions later in the complement cascade sequence, i.e., patients with lesions that block C5 functions, are found to be more prone only to Neisseria infection, and then only somewhat more prone (Fearon, 1983).

C3b also forms a complex with other components unique to each pathway to form classical or alternative C5 convertase, which cleaves C5 into C5a and C5b. C3 is thus regarded as the central protein in the complement reaction sequence since it is essential to both the alternative and classical pathways (Wurzner et al., 1991). This property of C3b is regulated by the serum protease Factor I, which acts on C3b to produce iC3b. While still functional as opsonin, iC3b cannot form an active C5 convertase.

C5 is a 190 kDa beta globulin found in normal serum at approximately 75 μg/mL (0.4 μM). C5 is glycosylated, with about 1.5-3 percent of its mass attributed to carbohydrate. Mature C5 is a heterodimer of a 999 amino acid 115 kDa alpha chain that is disulfide linked to a 656 amino acid 75 kDa beta chain. C5 is synthesized as a single chain precursor protein product of a single copy gene (Haviland et al., 1991). The cDNA sequence of the transcript of this gene predicts a secreted pro-C5 precursor of 1659 amino acids along with an 18 amino acid leader sequence.

The pro-C5 precursor is cleaved after amino acid 655 and 659, to yield the beta chain as an amino terminal fragment (amino acid residues +1 to 655) and the alpha chain as a carboxyl terminal fragment (amino acid residues 660 to 1658), with four amino acids deleted between the two.

C5a is cleaved from the alpha chain of C5 by either alternative or classical C5 convertase as an amino terminal fragment comprising the first 74 amino acids of the alpha chain (i.e., amino acid residues 660-733). Approximately 20 percent of the 11 kDa mass of C5a is attributed to carbohydrate. The cleavage site for convertase action is at or immediately adjacent to amino acid residue 733. A compound that would bind at or adjacent to this cleavage site would have the potential to block access of the C5 convertase enzymes to the cleavage site and thereby act as a complement inhibitor.

C5 can also be activated by means other than C5 convertase activity. Limited trypsin digestion (Minta and Man, 1977; Wetsel and Kolb, 1982) and acid treatment

(Yamamoto and Gewurz, 1978; Vogt et al., 1989) can also cleave C5 and produce active C5b.

C5a is another anaphylatoxin. C5b combines with C6, C7, and C8 to form the C5b-8 complex at the surface of the target cell. Upon binding of several C9 molecules, the membrane attack complex (MAC, C5b-9, terminal complement complex-TCC) is formed. When sufficient numbers of MACs insert into target cell membranes the openings they create (MAC pores) mediate rapid osmotic lysis of the target cells. Lower, non-lytic concentrations of MACs can produce other effects. In particular, membrane insertion of small numbers of the C5b-9 complexes into endothelial cells and platelets can cause deleterious cell activation. In some cases activation may precede cell lysis.

As mentioned above, C3a and C5a are anaphylatoxins. These activated complement components can trigger mast cell degranulation, which releases histamine and other mediators of inflammation, resulting in smooth muscle contraction, increased vascular permeability, leukocyte activation, and other inflammatory phenomena including cellular proliferation resulting in hypercellularity. C5a also functions as a chemotactic peptide that serves to attract pro-inflammatory granulocytes to the site of complement activation.

The beneficial effect of anti-C5 mAb has previously been reported in several experimental models including myocardial reperfusion (Vakeva et al., 1998), systemic lupus erythematosus (Wang et al., 1996) and rheumatoid arthritis (Wang et al., 1995); as well as in human clinical trials (Kirschfink, 2001) of autoimmune disease, cardiopulmonary bypass and acute myocardial infarction.

IV Measures of Quality of Life

Various measurements exist to assess quality of life and the effect of medical interventions on quality of life for example the Mini-Mental State Examination (MMSE), the Short Test of Mental Status, the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire, the FACIT questionnaires and subscales including fatigue and anemia, the Likert Scale, and Borg Scale (Tombaugh, et al., J. Am. Geriatr. Soc. 40:922, 1992; Cummings, JAMA. 269(18):2420, 1993; Crum, et al., JAMA. 269(18):2386, 1993; Folstein, et al., J. Psychiat. Res. 12:189,1975; Kokmen, et al., Mayo Clin. Proc. 62:281, 1987; Tang-Wai, et al., Arch. Neurol. 60:1777, 2003; Tamburini, Ann.

Oncol. 12(Suppl. 3):S7, 2001; Webster et al., Health and Quality of Life Outcomes. 1:79, 2003, www.hqlo.com/content/1/1/79; Grant, et al., Chest. 116:1208, 1999; and www.qolid.org). Any of these measurements may be used to assess the change in quality of life due to administration of a compound which inhibits complement or inhibits formation of C5b-9.

In certain embodiments, improvement in quality of life due to administration of a compound which inhibits complement or inhibits formation of C5b-9 is measured by the Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System. In certain embodiments, improvement in quality of life is measured by: a) full scales; b) stand-alone subscales; and c) symptom indices.

In certain embodiments, improvement in quality of life due to administration of a compound which inhibits complement or inhibits formation of C5b-9 is measured by a European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire. In certain embodiments, the EORTC questionnaire is the QLQ-C30.

In certain embodiments, improvement in quality of life is measured by the health-adjusted life expectancy (HALE) index as described in Wilkins, R. and Adams, OB., Am J Public Health, 73:1073-1080 (1983). Health-adjusted life expectancy is an average of the quality-adjusted life years (QALY) for a given population and can be used to evaluate the therapeutic value of a medical intervention. Quality-adjusted life years is a health index that weighs each year of life on a scale from 1 to 0 (Weinstein MC and Stason WB, N Engl J Med, 296:716-721 (1977)). Perfect health is rated as 1, death is rated as 0, and disability and pain are rated based on severity. QALY is determined by multiplying the number of years at each health status.

In certain embodiments, improvement in quality of life is measured by the following instruments: Years of potential life lost, Disability-free life expectancy, Health-adjusted life year, Quality adjusted life year, Healthy years equivalents, Healthy days gained, Episode-free day, Q-TWiST, Health Utilities Index, and Years of healthy life. These measurements account for both changes in mortality as well as changes in morbidity and disability. Any of these measurements may be used to assess the change in quality of life due to administration of a compound which inhibits complement or inhibits formation of C5b-9.

In one embodiment, the disclosed methods improve the quality of life of a patient for at least one day, at least one week, at least two weeks, at least three weeks, at least one

month, at least two months, at least three months, at least 6 months, at least one year, at least 18 months, at least two years, at least 30 months, or at least three years, or the duration of treatment.

In certain embodiments, the symptoms used to measure quality of life are scaled for intensity. In certain embodiments, the symptoms are scaled for frequency. In certain embodiments, the symptoms are scaled for intensity and frequency.

In certain aspects, the application provides a method for prolonging the health-adjusted life expectancy of a subject comprising administering to the subject a compound which inhibits complement or inhibits formation of C5b-9. The above measurements account for both changes in mortality as well as changes in morbidity and disability. Any of these measurements may be used to assess the change in quality-adjusted life expectancy due to administration of a compound which inhibits complement or inhibits formation of C5b-9.

In one embodiment, the disclosed methods prolong the health-adjusted life expectancy in a subject by at least one day, at least one week, at least two weeks, at least three weeks, at least one month, at least two months, at least three months, at least 6 months, at least one year, at least 18 months, at least two years, at least 30 months, or at least three years as measured by the health-adjusted life expectancy (HALE) index as described in Wilkins et al. Am J Public Health, 73:1073-1080 (1983). Health-adjusted life expectancy is an average of the quality-adjusted life years (QALY) for a given population and can be used to evaluate the therapeutic value of a medical intervention. Quality-adjusted life years is a health index that weighs each year of life on a scale from 1 to 0 (Weinstein et al., N Engl J Med, 296:716-721 (1977)). Perfect health is rated as 1, death is rated as 0, and disability and pain are rated based on severity. QALY is determined by multiplying the number of years at each health status.

V Inhibitors of the Complement Cascade

In certain embodiments, any compound which binds to or otherwise blocks the generation and/or activity of one or more complement components can be used in the present methods. In certain embodiments, a complement inhibitor may be a small molecule (up to 6,000 Da in molecular weight), a nucleic acid or nucleic acid analog, a peptidomimetic, or a macromolecule that is not a nucleic acid, a serine protease inhibitor, or a protein. These agents include, but are not limited to, small organic molecules, RNA

aptamers including ARC187 (which is commercially available from Archemix Corp., Cambridge, Mass.), L-RNA aptamers, Spiegelmers, antisense compounds, molecules which may be utilized in RNA interference (RNAi) such as double stranded RNA including small interfering RNA (siRNA), locked nucleic acid (LNA) inhibitors, peptide nucleic acid (PNA) inhibitors.

In certain embodiments, a complement inhibitor may be a protein or protein fragment. Proteins are known which inhibit the complement cascade, including CD59, CD55, CD46 and other inhibitors of C8 and C9 (see, e.g., U.S. Patent 6,100,443). Proteins known as complement receptors and which bind complement are also known (see, Published PCT Patent Application WO 92/10205 and U.S. Patent 6,057,131). Use of soluble forms of complement receptors, e.g., soluble CR1, can inhibit the consequences of complement activation such as neutrophil oxidative burst, complement mediated neural injury, and C3a and C5a production. In certain embodiments, a complement inhibitor may be naturally occurring or soluble forms of complement inhibitory compounds such as CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, compIestatin, and K76 COOH. Those of skill in the art recognize the above as some, but not all, of the known methods of inhibiting complement and its activation.

In certain embodiments, a complement inhibitor may be an antibody capable of inhibiting complement, such as an antibody that can block the formation of MAC. For example, an antibody complement inhibitor may include an antibody that binds C5. Such anti-C5 antibodies may directly interact with C5 and/or C5b, so as to inhibit the formation of and/or physiologic function of C5b.

Suitable anti-C5 antibodies are known to those of skill in the art. Antibodies can be made to individual components of activated complement, e.g., antibodies to C7, C9, etc. (see, e.g., U.S. Patent 6,534,058; published U.S. patent application US 2003/0129187; and U.S. Patent 5,660,825). U.S. Patent 6,355,245 teaches an antibody which binds to C5 and inhibits cleavage into C5a and C5b thereby decreasing the formation not only of C5a but also the downstream complement components.

The concentration and/or physiologic activity of C5a and C5b in a body fluid can be measured by methods well known in the art. For C5a such methods include chemotaxis assays, RIAs, or ELISAs (see, for example, Ward and Zvaifler, J Clin Invest. 1971 Mar;50(3):606-16; Wurzner, et al., Complement Inflamm. 8:328-340, 1991). For C5b, hemolytic assays or assays for soluble C5b-9 as discussed herein can be used. Other assays

known in the art can also be used. Using assays of these or other suitable types, candidate antibodies capable of inhibiting complement such as anti-C5 antibodies, now known or subsequently identified, can be screened in order to 1) identify compounds that are useful in the practice of the application and 2) determine the appropriate dosage levels of such compounds.

An antibody capable of inhibiting complement such as an antibody that binds C5 affecting C5b is preferably used at concentrations providing substantial reduction (i.e., reduction by at least about 25% as compared to that in the absence of the antibody that binds C5) in the C5b levels present in at least one blood-derived fluid of the patient following activation of complement within the fluid. Such concentrations can be conveniently determined by measuring the cell-lysing ability (e.g., hemolytic activity) of complement present in the fluid or the levels of soluble C5b-9 present in the fluid. Accordingly, a specific concentration for an antibody that affects C5b is one that results in a substantial reduction (i.e., a reduction by at least about 25%) in the cell-lysing ability of the complement present in at least one of the patient's blood-derived fluids. Reductions of the cell-lysing ability of complement present in the patient's body fluids can be measured by methods well known in the art such as, for example, by a conventional hemolytic assay such as the hemolysis assay described by Kabat and Mayer (eds), "Experimental Immunochemistry, 2d Edition", 135-240, Springfield, IL, CC Thomas (1961), pages 135-139, or a conventional variation of that assay such as the chicken erythrocyte hemolysis method described below.

Specific antibodies capable of inhibiting complement, such as an antibody that binds C5, are relatively specific and do not block the functions of early complement components. In particular, such specific agents will not substantially impair the opsonization functions associated with complement component C3b, which functions provide a means for clearance of foreign particles and substances from the body.

C3b is generated by the cleavage of C3, which is carried out by classical and/or alternative C3 convertases and results in the generation of both C3a and C3b. Therefore, in order not to impair the opsonization functions associated with C3b, specific antibodies capable of inhibiting complement such as an antibody that binds C5 do not substantially interfere with the cleavage of complement component C3 in a body fluid of the patient (e.g., serum) into C3a and C3b. Such interference with the cleavage of C3 can be detected by measuring body fluid levels of C3a and/or C3b, which are produced in equimolar ratios

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by the actions of the C3 convertases. Such measurements are informative because C3a and C3b levels will be reduced (compared to a matched sample without the antibody capable of inhibiting complement such as an antibody that binds C5) if cleavage is interfered with by an antibody capable of inhibiting complement such as an antibody that binds C5.

In practice, the quantitative measurement of such cleavage is generally more accurate when carried out by the measurement of body fluid C3a levels rather than of body fluid C3b levels, since C3a remains in the fluid phase whereas C3b is rapidly cleared. C3a levels in a body fluid can be measured by methods well known in the art such as, for example, by using a commercially available C3a EIA kit, e.g., that sold by Quidel Corporation, San Diego, Calif., according to the manufacturer's specifications. Particularly specific antibodies capable of inhibiting complement such as an antibody that binds C5 produce essentially no reduction in body fluid C3a levels following complement activation when tested in such assays.

Certain antibodies of the disclosure will prevent the cleavage of C5 to form C5a and C5b, thus preventing the generation of the anaphylatoxic activity associated with C5a and preventing the assembly of the membrane attack complex associated with C5b. As discussed above, in a particular embodiment, these anti-C5 antibodies will not impair the opsonization function associated with the action of C3b.

A preferred method of inhibiting complement activity is to use a monoclonal antibody which binds to complement C5 and inhibits cleavage. This decreases the formation of both C5a and C5b while at the same time allowing the formation of C3a and C3b which are beneficial to the recipient. Such antibodies which are specific to human complement are known (U.S. Patent 6,355,245). These antibodies disclosed in U.S. Patent 6,355,245 include a preferred whole antibody (now named eculizumab). A similar antibody against mouse C5 is called BB5.1 (Frei et al., Mol. Cell. Probes. 1:141-149 (1987)). Antibodies to inhibit complement activity need not be monoclonal antibodies. They can be, e.g., polyclonal antibodies. They may additionally be antibody fragments. An antibody fragment includes, but is not limited to, an Fab, F(ab'), $F(ab')_2$, single-chain antibody, and Fv. Furthermore, it is well known by those of skill in the art that antibodies can be humanized (Jones et al., Nature 321:522-5 (1986)), chimerized, or deimmunized. The antibodies to be used in the present disclosure may be any of these. It is preferable to use humanized antibodies.

In specific embodiments, a therapeutic agent of the disclosure comprises an antibody or antibody fragment. Antibodies and fragments thereof may be made by any conventional method, such as those methods described herein. Antibodies are found in multiple forms, e.g., IgA, IgG, IgM, etc. Additionally, antibodies can be engineered in numerous ways. They can be made as single-chain antibodies (including small modular immunopharmaceuticals or SMIPs™), Fab and $F(ab')_2$ fragments, etc. Antibodies can be humanized, chimerized, deimmunized, or fully human. Numerous publications set forth the many types of antibodies and the methods of engineering such antibodies. For example, see U.S. Patent Nos. 6,355,245; 6,180,370; 5,693,762; 6,407,213; 6,548,640; 5,565,332; 5,225,539; 6,103,889; and 5,260,203.

This invention provides fragments of anti-C5 antibodies, which may comprise a portion of an intact antibody, preferably the antigen-binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8:1057-1062 (1995)); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

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 of an antibody yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

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

free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

"Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of an 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 that enables the scFv to form the desired structure for antigen binding. For a review of scFv see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore, eds. (Springer-Verlag: New York, 1994), pp. 269-315.

SMIPs are a class of single-chain peptide engineered to include a target binding region, effector domain (CH2 and CH3 domains). *See, e.g.*, U.S. Patent Application Publication No. 20050238646. The target binding region may be derived from the variable region or CDRs of an antibody, e.g., an antibody that binds C5 of the application. Alternatively, the target binding region is derived from a protein that binds C5.

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).

It is well known that the binding to a molecule (or a pathogen) of antibodies with an Fc region assists in the processing and clearance of the molecule (or pathogen). The Fc portions of antibodies are recognized by specialized receptors expressed by immune effector cells. The Fc portions of IgG1 and IgG3 antibodies are recognized by Fc receptors present on the surface of phagocytic cells such as macrophages and neutrophils, which can thereby bind and engulf the molecules or pathogens coated with antibodies of these isotypes (C. A. Janeway et al., Immunobiology 5th edition, page 147, Garland Publishing (New York, 2001)).

This disclosure also provides monoclonal anti-C5 antibodies. A monoclonal antibody can be 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 that 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 often synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. Monoclonal antibodies may also be produced in transfected cells, such as CHO cells and NS0 cells. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies and does not require production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by the hybridoma method first described by Kohler et al., Nature 256:495-497 (1975), or may be made by recombinant DNA methods (*see, e.g.*, U.S. Patent Nos. 4,816,567 and 6,331,415). 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.

A description of the preparation of a mouse anti-human-C5 monoclonal antibody with specific binding characteristics is presented in U.S. Patent Application Publication No. 20050226870. Wurzner et al., Complement Inflamm. 8:328-340 (1991), describe the preparation of other mouse anti-human-C5 monoclonal antibodies referred to as N19-8 and N20-9.

Other antibodies specifically contemplated are "oligoclonal" antibodies. As used herein, the term "oligoclonal" antibodies" refers to a predetermined mixture of distinct monoclonal antibodies. *See, e.g.*, PCT publication WO 95/20401; U.S. Patent Nos. 5,789,208 and 6,335,163. In one embodiment, oligoclonal antibodies consisting of a predetermined mixture of antibodies against one or more epitopes are generated in a single cell. In other embodiments, oligoclonal antibodies comprise a plurality of heavy chains capable of pairing with a common light chain to generate antibodies with multiple specificities (*e.g.*, PCT publication WO 04/009618). Oligoclonal antibodies are particularly useful when it is desired to target multiple epitopes on a single target molecule (*e.g.*, C5). In view of the assays and epitopes disclosed herein, those skilled in the art can generate or select antibodies or mixtures of antibodies that are applicable for an intended purpose and desired need.

In certain embodiments that include a humanized and/or chimeric antibody, one or more of the CDRs are derived from an anti-human C5 antibody. In a specific embodiment, all of the CDRs are derived from an anti-human C5 antibody. In another specific embodiment, the CDRs from more than one anti-human C5 antibody are mixed and matched in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first anti-human C5 antibody combined with CDR2 and CDR3 from the light chain of a second anti-human C5 antibody, and the CDRs from the heavy chain may be derived from a third anti-human C5 antibody. Further, the framework regions may be derived from one of the same anti-human C5 antibodies, from one or more different antibodies, such as a human antibody, or from a humanized antibody. Human or humanized antibodies are specific for administration to human patients.

In certain embodiments, single chain antibodies, and chimeric, humanized or primatized (CDR-grafted) antibodies, as well as chimeric or CDR-grafted single chain antibodies, comprising portions derived from different species, are also encompassed by the present disclosure as antigen-binding fragments of an antibody. The various portions of these antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or humanized chain can be expressed to produce a contiguous protein. See, e.g., U.S. Pat. Nos. 4,816,567 and 6,331,415; U.S. Pat. No. 4,816,397; European Patent No. 0,120,694; WO 86/01533; European Patent No. 0,194,276 B1; U.S. Pat. No. 5,225,539; and European Patent No. 0,239,400 B1. See also, Newman et al., BioTechnology 10:1455-1460 (1992), regarding primatized antibody. See, e.g., Ladner et al., U.S. Pat. No. 4,946,778; and Bird et al., Science 242:423-426 (1988), regarding single chain antibodies.

In addition, functional fragments of antibodies, including fragments of chimeric, humanized, primatized or single chain antibodies, can also be produced. Functional fragments of the subject antibodies retain at least one binding function and/or modulation function of the full-length antibody from which they are derived. Preferred functional fragments retain an antigen-binding function of a corresponding full-length antibody (such as for example, ability of antibody that binds C5 to bind C5).

General methods for the immunization of animals (in this case with C5 and/or C5b, etc.), isolation of antibody producing cells, fusion of such cells with immortal cells (e.g., myeloma cells) to generate hybridomas secreting monoclonal antibodies, screening of

hybridoma supernatants for reactivity of secreted monoclonal antibodies with a desired antigen (in this case the immunogen or a molecule containing the immunogen), the preparation of quantities of such antibodies in hybridoma supernatants or ascites fluids, and for the purification and storage of such monoclonal antibodies, can be found in numerous publications. These include: Coligan, et al., eds. Current Protocols In Immunology, John Wiley & Sons, New York, 1992; Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1988; Liddell and Cryer, A Practical Guide To Monoclonal Antibodies, John Wiley & Sons, Chichester, West Sussex, England, 1991; Montz et al., Cellular Immunol. 127:337-351 (1990); Wurzner et al., Complement Inflamm. 8:328-340 (1991); and Mollnes et al., Scand. J. Immunol. 28:307-312 (1988).

VI Methods of Treatment

Methods of the application may be used to treat paroxysmal nocturnal hemoglobinuria associated symptoms. Methods of the application may be used to treat anemia associated symptoms. Treatment of paroxysmal nocturnal hemoglobinuria and/or anemia may be administered by standard means. Treatments of the application may be used in combination with other treatments of the application or known treatments for paroxysmal nocturnal hemoglobinuria and/or anemia. Treatments of the application may be co-administered with other treatments that treat symptoms of paroxysmal nocturnal hemoglobinuria and/or anemia.

VII Pharmaceutical Formulations and Uses

Methods of administration of small molecules, proteins, and nucleic acids are well-known to those of skill in the art. Methods of administration of antibodies are well-known to those of skill in the art. To achieve the desired inhibition, the antibodies can be administered in a variety of unit dosage forms. The dose will vary according to the particular antibody. For example, different antibodies may have different masses and/or affinities, and thus require different dosage levels. Antibodies prepared as Fab fragments will also require differing dosages than the equivalent intact immunoglobulins, as they are of considerably smaller mass than intact immunoglobulins, and thus require lower dosages to reach the same molar levels in the patient's blood. The dose will also vary depending on the manner of administration, the particular symptoms of the patient being treated, the overall health, condition, size, and age of the patient, and the judgment of the prescribing

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physician. Dosage levels of the antibodies for human subjects are generally between about 1 mg per kg and about 100 mg per kg per patient per treatment, and preferably between about 5 mg per kg and about 50 mg per kg per patient per treatment. In terms of plasma concentrations, the antibody concentrations are preferably in the range from about 25 μg/mL to about 500 μg/mL. However, greater amounts may be required for extreme cases and smaller amounts may be sufficient for milder cases.

In certain embodiments, the pharmaceutical composition is in a single unit dosage form. In certain embodiments, the single unit dosage form is a 300 mg unit dosage form. In certain embodiments, the pharmaceutical composition is lyophilized. In certain embodiments, the pharmaceutical composition is a sterile solution. In certain embodiments, the pharmaceutical composition is a preservative free formulation. In certain embodiments, the pharmaceutical composition comprises a 300 mg single-use formulation of 30 ml of a 10 mg/ml sterile, preservative free solution. In certain embodiments, the antibody is administered according to the following protocol: 600 mg via 25 to 45 minute IV infusion every 7 ± 2 days for the first 4 weeks, followed by 900 mg for the fifth dose 7 ± 2 days later, then 900 mg every 14 ± 2 days thereafter. Antibody is administered via IV infusion over 25 to 45 minute.

Administration of the anti-C5 antibodies will generally be performed by an intravascular route, e.g., via intravenous infusion by injection. Other routes of administration may be used if desired but an intravenous route will be the most preferable. Formulations suitable for injection are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed. (1985). Such formulations must be sterile and non-pyrogenic, and generally will include a pharmaceutically effective carrier, such as saline, buffered (e.g., phosphate buffered) saline, Hank's solution, Ringer's solution, dextrose/saline, glucose solutions, and the like. The formulations may contain pharmaceutically acceptable auxiliary substances as required, such as, tonicity adjusting agents, wetting agents, bactericidal agents, preservatives, stabilizers, and the like. In certain embodiments, complement inhibitors such as eculizumab may be administered via IV infusion and diluted to a final concentration of 5 mg/ml prior to administration.

Administration of the antibodies capable of inhibiting complement such as an antibody that binds C5 will generally be performed by a parenteral route, typically via injection such as intra-articular or intravascular injection (e.g., intravenous infusion) or intramuscular injection. Other routes of administration, e.g., oral (p.o.), may be used if

desired and practicable for the particular antibody capable of inhibiting complement to be administered. Antibodies capable of inhibiting complement such as an antibody that binds C5 can also be administered in a variety of unit dosage forms and their dosages will also vary with the size, potency, and in vivo half-life of the particular antibody capable of inhibiting complement being administered. Doses of antibodies capable of inhibiting complement such as an antibody that binds C5 will also vary depending on the manner of administration, the particular symptoms of the patient being treated, the overall health, condition, size, and age of the patient, and the judgment of the prescribing physician.

In certain embodiments, a typical therapeutic treatment includes a series of doses, which will usually be administered concurrently with the monitoring of clinical endpoints with the dosage levels adjusted as needed to achieve the desired clinical outcome. In certain embodiments, treatment is administered in multiple dosages over at least a week. In certain embodiments, treatment is administered in multiple dosages over at least a month. In certain embodiments, treatment is administered in multiple dosages over at least a year. In certain embodiments, treatment is administered in multiple dosages over the remainder of the patient's life.

The frequency of administration may also be adjusted according to various parameters. These include the clinical response, the plasma half-life of the therapeutic of the disclosure, and the levels of the antibody in a body fluid, such as, blood, plasma, serum, or synovial fluid. To guide adjustment of the frequency of administration, levels of the therapeutic of the disclosure in the body fluid may be monitored during the course of treatment.

In certain embodiments, the frequency of administration may be adjusted according to an assay measuring cell-lysing ability of complement present in one or more of the patient's body fluids. The cell-lysing ability can be measured as percent hemolysis in hemolytic assays of the types described herein. A 10% or 25% or 50% reduction in the cell-lysing ability of complement present in a body fluid after treatment with the antibody capable of inhibiting complement used in the practice of the application means that the percent hemolysis after treatment is 90, 75, or 50 percent, respectively, of the percent hemolysis before treatment.

For the treatment of hemolytic diseases such as PNH by systemic administration of an antibody capable of inhibiting complement such as an antibody that binds C5 (as opposed to local administration), administration of a large initial dose is specific, i.e., a

single initial dose sufficient to yield a substantial reduction, and more preferably an at least about 50% reduction, in the hemolytic activity of the patient's serum. Such a large initial dose is preferably followed by regularly repeated administration of tapered doses as needed to maintain substantial reductions of serum hemolytic titer. In another embodiment, the initial dose is given by both local and systemic routes, followed by repeated systemic administration of tapered doses as described above.

Formulations particularly useful for antibody-based therapeutic agents are also described in U.S. Patent App. Publication Nos. 20030202972, 20040091490 and 20050158316. In certain embodiments, the liquid formulations of the application are substantially free of surfactant and/or inorganic salts. In another specific embodiment, the liquid formulations have a pH ranging from about 5.0 to about 7.0. In yet another specific embodiment, the liquid formulations comprise histidine at a concentration ranging from about 1 mM to about 100 mM. In still another specific embodiment, the liquid formulations comprise histidine at a concentration ranging from 1 mM to 100 mM. It is also contemplated that the liquid formulations may further comprise one or more excipients such as a saccharide, an amino acid (e.g., arginine, lysine, and methionine) and a polyol. Additional descriptions and methods of preparing and analyzing liquid formulations can be found, for example, in PCT publications WO 03/106644, WO 04/066957, and WO 04/091658.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the pharmaceutical compositions of the application.

In certain embodiments, formulations of the subject antibodies are pyrogen-free formulations which are substantially free of endotoxins and/or related pyrogenic substances. Endotoxins include toxins that are confined inside microorganisms and are released when the microorganisms are broken down or die. Pyrogenic substances also include fever-inducing, thermostable substances (glycoproteins) from the outer membrane of bacteria and other microorganisms. Both of these substances can cause fever, hypotension and shock if administered to humans. Due to the potential harmful effects, it is advantageous to remove even low amounts of endotoxins from intravenously administered pharmaceutical drug solutions. The Food & Drug Administration ("FDA") has set an upper limit of 5 endotoxin units (EU) per dose per kilogram body weight in a

single one hour period for intravenous drug applications (The United States Pharmacopeial Convention, Pharmacopeial Forum 26 (1):223 (2000)). When therapeutic proteins are administered in amounts of several hundred or thousand milligrams per kilogram body weight, as can be the case with monoclonal antibodies, it is advantageous to remove even trace amounts of endotoxin.

Formulations of the subject antibodies include those suitable for oral, dietary, topical, parenteral (e.g., intravenous, intraarterial, intramuscular, subcutaneous injection), ophthalmologic (e.g., topical or intraocular), inhalation (e.g., intrabronchial, intranasal or oral inhalation, intranasal drops), rectal, and/or intravaginal administration. Other suitable methods of administration can also include rechargeable or biodegradable devices and controlled release polymeric devices. Stents, in particular, may be coated with a controlled release polymer mixed with an agent of the application. The pharmaceutical compositions of this disclosure can also be administered as part of a combinatorial therapy with other agents (either in the same formulation or in a separate formulation).

The amount of the formulation which will be therapeutically effective can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems. The dosage of the compositions to be administered can be determined by the skilled artisan without undue experimentation in conjunction with standard dose-response studies. Relevant circumstances to be considered in making those determinations include the condition or conditions to be treated, the choice of composition to be administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms. For example, the actual patient body weight may be used to calculate the dose of the formulations in milliliters (mL) to be administered. There may be no downward adjustment to "ideal" weight. In such a situation, an appropriate dose may be calculated by the following formula: Dose (mL) = [patient weight (kg) x dose level (mg/kg)/ drug concentration (mg/mL)]

To achieve the desired treatment results, anti-C5 antibodies can be administered in a variety of unit dosage forms. The dose will vary according to the particular antibody. For example, different antibodies may have different masses and/or affinities, and thus require

different dosage levels. Antibodies prepared as Fab' fragments or single chain antibodies will also require differing dosages than the equivalent native immunoglobulins, as they are of considerably smaller mass than native immunoglobulins, and thus require lower dosages to reach the same molar levels in the patient's blood.

Other therapeutics of the disclosure can also be administered in a variety of unit dosage forms and their dosages will also vary with the size, potency, and *in vivo* half-life of the particular therapeutic being administered.

Doses of therapeutics of the disclosure will also vary depending on the manner of administration, the particular symptoms of the patient being treated, the overall health, condition, size, and age of the patient, and the judgment of the prescribing physician.

The formulations of the application can be distributed as articles of manufacture comprising packaging material and a pharmaceutical agent which comprises the antibody capable of inhibiting complement and a pharmaceutically acceptable carrier as appropriate to the mode of administration. The packaging material may include a label which indicates that the formulation is for use in the treatment of hemolytic diseases such as PNH. Although antibodies are preferred, especially anti-C5 antibodies which have already been shown to be safe and effective at decreasing the accumulation of downstream complement components in persons, the use of other complement inhibitors is also contemplated by this disclosure. The pharmaceutical formulations and uses of the disclosure may be combined with any known complement inhibitors or hemolytic diseases treatments known in the art.

In certain aspects, the application provides kits comprising a pharmaceutical composition of the application. In some embodiments, the kit further comprises at least one component of a closed sterile system. Components of the closed sterile system include, but are not limited to, needles, syringes, catheter based syringes, needle based injection devices, needle-less injection devices, filters, tubing, valves and cannulas. In a related embodiment, the kit comprise components for the removal of a preservative from the composition. Such components include filters, syringes, vials, containers, tubing, etc.

EXEMPLIFICATION

METHODS

Patient Selection

The TRIUMPH trial consisted of a 2-week screening period, an observation period of up to 3 months duration, and a 26-week treatment period.

During the screening period, patients were evaluated with respect to inclusion and exclusion criteria. Men and women, 18 years or older, diagnosed as having PNH with a type III erythrocyte population of ≥10%, and who had received at least 4 transfusions in the previous 12 months were eligible. Concomitant administration of erythropoietin, immunosuppressants, corticosteroids, coumadin, low molecular weight heparin, iron supplements, and folic acid were not reasons for exclusion, provided the doses were steady prior to the first visit and throughout the duration of the study. Because of the increased frequency of neisserial infections in individuals genetically deficient in terminal complement proteins, all patients were vaccinated against *Neisseria meningitidas*. Patients were to avoid conception. The protocol was approved by an Investigational Review Board at each clinical site and written informed consent was obtained from all patients enrolled.

Patients transfused with a mean pre-transfusion hemoglobin level >10.5 g/dL over the previous 12 months, and those who showed evidence of having a suppressed immune response, complement deficiency, or active bacterial infection, including any history of meningococcal disease, were excluded from the study. Patients were also not eligible if they had previously received a bone marrow transplant or if they had participated in another trial or received another investigational drug within 30 days of the first visit. An individualized transfusion algorithm was calculated for each patient based on their prior 12-month transfusion history; the written algorithm documented the number of packed red blood cell (PRBC) units transfused for given hemoglobin values and served as a prospectively determined guide for transfusion during observation and treatment periods.

Each patient considered eligible entered an observation period of up to 13 weeks in order to confirm their PBRC transfusion dependence. At least one transfusion --termed the "qualifying" transfusion-- during the 13-week observation period at a hemoglobin value at or below 9 g/dL with symptoms, or at or below 7 g/dL with or without symptoms, in accordance with the transfusion algorithm indicated for each patient, was a requirement to

proceed to randomization. The hemoglobin value at which each individual's qualifying transfusion was administered, was defined as the hemoglobin "set point" for that individual for the purpose of the primary efficacy variable. A platelet count ≥100,000/mL and a LDH level ≥1.5 times the upper limit of the normal range were also required either at screening or during the observation period for eligibility.

Study Design

Patients were randomly assigned on a one-on-one basis to receive either placebo or eculizumab (Soliris™, Alexion Pharmaceuticals, Inc.) within 10 days of the qualifying transfusion. Study medication was dosed in a blinded fashion as follows: 600 mg eculizumab for patients randomly assigned to active drug, or placebo for those patients randomly assigned to placebo, respectively via IV infusion every 7±1 days for 4 doses; followed by 900 mg eculizumab, or placebo, respectively, via IV infusion 7±1 day later; followed by a maintenance dose of 900 mg eculizumab, or placebo, respectively, via IV infusion every 14±2 days for a total of 26 weeks of treatment.

Measures of Clinical Efficacy

There were two co-primary endpoints in the study: (1) stabilization of hemoglobin levels, defined as a hemoglobin value maintained above the individual hemoglobin set point in the absence of transfusions for the entire 26-week treatment period, and (2) reduction in units of PRBCs transfused during the 26-week treatment phase of the study. The trigger for transfusion during the study period remained unchanged for each patient, as compared with their care before entry into the study: patients received blood transfusions when they had symptoms resulting from anemia and reached their individualized, pre-determined "set point". Pre-specified secondary endpoints included transfusion avoidance, hemolysis as measured by LDH area under the curve from baseline to 26 weeks, and QoL changes as measured from baseline to 26 weeks using the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) instrument.[13] Pre-specified exploratory analyses included assessment of the EORTC QLQ-C30 instrument,[14] the change in LDH from baseline through week 26, and thrombosis. Other pre-specified measurements included pharmacokinetics, pharmacodynamics, and immunogenicity of eculizumab. Time to first transfusion during the 26-week treatment phase and the proportion of PNH type III blood cells were also assessed.

Safety Assessments

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Treatment-emergent adverse events, clinical laboratory tests (e.g., serum chemical analyses and complete blood counts), electrocardiogram data, and vital signs were assessed. Adverse events were defined using the MedDRA preferred terms and tabulated as incidence rates per treatment group.

Statistical Analysis

For co-primary endpoints, analyses were performed according to the intention to treat using the data from all patients who were randomized and received study drug; stabilization of hemoglobin levels was analyzed using the Fisher's exact test and total PRBC units transfused were analyzed with the Wilcoxon's rank sum test. For comparison of treatment effect on transfusion avoidance, the Fisher's exact test was used on the incidence and the log rank test was used for time to first transfusion. For LDH area under the curve the Wilcoxon's rank sum test was used.

Quality of life measure of fatigue was assessed using the scoring guidelines for the FACIT-Fatigue instrument.[15] Assessment of quality of life measures based on the EORTC QLQ-C30 instrument was conducted in accordance with the appropriate scoring guidelines.[16] The changes of FACIT-Fatigue and EORTC QLQ-C30 scores from baseline through 26 weeks were analyzed using a mixed model, with baseline as covariate, treatment and time as fixed effects, and patient as a random effect. Changes in LDH levels and PNH type III erythrocytes from baseline through 26 weeks were analyzed using the same mixed model. Two-sided tests were used for all analyses. The adverse events and long-term safety checklist were tabulated separately and compared between treatments using the Fisher's exact test. A p-value ≤ 0.05 was considered to be statistically significant.

RESULTS

Characteristics of Patients

A total of 115 PNH patients were screened. Six patients did not meet the inclusion/exclusion criteria during the screening period. Twenty-one other patients did not receive a qualifying transfusion and were not randomized into the treatment phase. One patient who did not meet the inclusion criteria was inadvertently randomized, but did not receive study medication. Thus 87 hemolytic PNH patients (35 men and 52 women) were enrolled and randomized to receive either eculizumab (N=43) or placebo (N=44), exceeding the original target of 75 randomized patients.

Patient characteristics were similar in the eculizumab- and placebo-treated cohorts; median age, 41 (range 20-85) and 35 (range 18-78) years; median duration of PNH, 4.2 (range 0.8 to 29.7) and 9.2 (range 0.4 to 38.3) years; patients with history of aplastic anemia, 4 and 11; history of myelodysplastic syndrome, 1 and 0; and history of thrombosis, 9 (16 events) and 8 (11 events). Stable usage of concomitant medications at baseline in the eculizumab- and placebo-treated groups included the following: erythropoietin, 3 patients and 0 patients; cyclosporine, 1 and 1; anticoagulants (coumarins or heparins) 21 and 11; and steroids (glucocorticoids or androgenic steroids), 12 and 12, respectively.

Of the 87 patients randomized, 85 completed the trial. Two patients who did not complete the trial had been randomized to the eculizumab arm: one patient discontinued due to the inconvenience of travel to the study site and the second patient became pregnant. Ten patients in the placebo-treatment group discontinued infusions, in all cases due to perceived lack of efficacy, but they remained in the study for monitoring purposes.

Pharmacokinetics/Pharmacodynamics

In 42 of the 43 eculizumab-treated patients the levels of drug during the maintenance period (900 mg every 2 weeks±2 days) were sufficient to completely block serum hemolytic activity (mean trough value at week 26 of 101.8 μg/mL). A single patient did not sustain therapeutic trough levels of eculizumab and demonstrated a breakthrough in complement blockade during the last few days of each dosing interval. These breakthroughs were clinically manageable and quickly resolved following the next dose.

Hemolytic Efficacy Variables

The impact of terminal complement inhibition with eculizumab on chronic intravascular hemolysis in PNH patients was demonstrated in this study by an immediate (one week) and sustained decrease in mean levels of LDH (Figure 1A). The median LDH area under the curve during the 26-week study period was reduced 85.8% in eculizumab-relative to placebo-treated patients ($P<0.001$). The mean LDH level decreased from 2199.7±157.7 IU/L at baseline to 327.3±67.6 IU/L by 26 weeks in eculizumab-treated patients while levels in placebo-treated patients remained consistently elevated with values of 2259.0±158.5 IU/L at baseline and 2418.9±140.3 IU/L at 26 weeks ($P<0.001$, for eculizumab versus placebo). A second biochemical measure of hemolysis, serum aspartate aminotransferase (AST), also showed a statistically significant improvement following eculizumab- versus placebo-treatment (data not shown). Haptoglobin levels were statistically significantly increased in eculizumab- as compared to placebo-treated patients

but mean levels of haptoglobin were still below normal levels in eculizumab-treated patients (data not shown).

Figure 1A shows the degree of intravascular hemolysis in PNH patients, demonstrated by mean lactate dehydrogenase (LDH) levels (±standard error) from baseline (study week 0) to week 26 for both eculizumab- and placebo-treated patient populations. Screening occurred up to 3 months prior to study week 0. The upper limit of the normal range (103-223 IU/L) for LDH is indicated by a dashed line. LDH was reduced to a mean level just above the upper limit of normal at week 26 for eculizumab-treated patients; 15 of 41 patients who completed the study demonstrated LDH levels within the normal range. All placebo-treated patients remained at least 5 times above the upper limit of normal at week 26. The P value is based on a mixed model analysis from baseline through week 26. Figure 1B shows the mean proportion (±standard error) of PNH type III erythrocytes assessed for placebo- and eculizumab-treated patients. The screening visit occurred up to 3 months prior to study week 0. The P value is based on a mixed model analysis from baseline through week 26.

A corollary to the reduction in intravascular hemolysis during eculizumab treatment was an observed increase in the PNH type III erythrocyte population (Figure 1B). The mean proportions of type III erythrocytes increased from 28.1±2.0% at baseline to 56.9±3.6% by week 26 for eculizumab-treated patients while proportions in the placebo group remained constant with mean values of 35.7±2.8% before treatment to 35.5±2.8% at 26 weeks ($P<0.001$, for eculizumab versus placebo). By contrast, the proportions of PNH type III granulocytes and monocytes did not change significantly between the treatment groups during the treatment period and were greater than 90% at week 26.

CLINICAL EFFICACY

Co-primary endpoints

The co-primary efficacy endpoints in the TRIUMPH trial were stabilization of hemoglobin levels and reduction in PRBC units transfused. At the end of the treatment period, 48.8% of eculizumab-treated patients had maintained levels of hemoglobin above the pre-specified set-point (median set-point value of 7.7 g/dL for both treatment groups) in the absence of transfusions, whereas stabilization of hemoglobin did not occur in any of the patients in the placebo group ($P<0.001$; Table 1). By week 26, the median of PRBC units transfused per patient was 0 in the eculizumab group and 10.0 in the placebo cohort

(P<0.001), while the mean of PRBC units transfused was 3.0 and 11.0 in the eculizumab and placebo cohorts, respectively. In the 6-month period prior to the study, the median of PRBC units transfused per patient was 9.0 in the eculizumab cohort and 8.5 in placebo patients while the mean of PRBC units transfused was 9.6±0.6 and 9.7±0.7, respectively. Mean hemoglobin levels were similar between the treatment groups at baseline (10.0±1.8 g/dL in eculizumab-treated patients and 9.7±1.8 g/dL in placebo-treated patients) and did not substantially change by week 26 (10.1±2.5 g/dL and 8.9±2.2 g/dL in eculizumab and placebo cohorts, respectively).

The median time to first transfusion was not reached during the study period in eculizumab-treated patients (it was greater than 26 weeks) while the placebo group reached the median time to first transfusion in only 4 weeks (P<0.001; Figure 2). Transfusion avoidance was achieved in 51.2% and 0% of the eculizumab and placebo cohorts, respectively (P<0.001). By the end of the 26-week treatment period, the total PRBC units transfused were 131 in eculizumab-treated patients versus 482 in the placebo group (Table 1). By contrast, in the 6-month period prior to the study, total PRBC units transfused in the eculizumab- and placebo-cohorts were 413 and 417, respectively.

Table 1

Stabilization of Hemoglobin Levels and Reduction in Transfusion Requirements during Eculizumab Treatment

	PRE-TREATMENT HISTORY*		TREATMENT PERIOD		
	Placebo	Eculizumab	Placebo	Eculizumab	P Value
†Stabilization of Hemoglobin Levels in the absence of transfusions (percent of patients)	NA	NA	0	48.8	<0.001‡
†Units Transfused per Patient					
Median	8.5	9.0	10	0	<0.001§
Mean±SE	9.7±0.7	9.6±0.6	11.0±0.83	3.0±0.67	
Total Units Transfused per Group	417	413	482	131	

*Twelve month historical transfusion data normalized to 6 months

†Co-primary endpoints

‡Base on 2-sided Fisher's exact test

§Based on Wilcoxon's rank sum test

NA, not applicable

Improvements in quality of life measures

Assessments of quality of life in PNH patients during eculizumab treatment were performed using two different instruments, FACIT-Fatigue and the EORTC QLQ-C30. Eculizumab-treated patients showed a mean increase (improvement) in the FACIT-Fatigue score of 6.4±1.2 points from baseline to 26-weeks while the mean score in placebo patients decreased by 4.0±1.7 points, a total difference between the treatment groups of 10.4 points (Figure 3). Mixed model analysis of covariance demonstrated a statistically significant difference between treatment groups (P<0.001).

For the EORTC instrument, improvements were observed with eculizumab-treatment in each subscale. Statistically significant improvements with eculizumab-compared with placebo-treated groups were observed in the following quality of life

subscales (Table 2): global health status (P<0.001), physical functioning (P<0.001), emotional functioning (P=0.008), cognitive functioning (P=0.002), role functioning (P<0.001), social functioning (P=0.003), fatigue (P<0.001), pain (P=0.002), dyspnea (P<0.001), appetite loss (P<0.001), and insomnia (P=0.014). The improvements with eculizumab treatment in the other scales, including nausea and vomiting, diarrhea, constipation, and financial difficulties, did not reach statistical significance.

Table 2

Effect of Eculizumab Treatment on

Quality of Life Assessed by the EORTC QLQ-C30 Instrument

	Mean Change from Baseline to Week 26*			
	Placebo	Eculizumab	Absolute Difference	P Value†
Global Health Status	-8.5	10.9	19.4	<0.001
Functional				
Role	-6.9	17.9	24.8	<0.001
Social	2.0	16.7	14.6	=0.003
Cognitive	-6.1	7.9	14.0	=0.002
Physical	-3.5	9.4	13.0	<0.001
Emotional	-3.7	7.5	11.2	=0.008
Symptoms/Single Items				
Fatigue	10.0	-16.9	27.0	<0.001
Pain	5.3	-12.3	17.6	=0.002
Dyspnea	8.9	-7.9	16.9	<0.001
Appetite Loss	3.3	-10.3	13.6	<0.001
Insomnia	4.9	-7.9	12.8	=0.014

Financial Difficulties	0.0	-10.3	10.3	=0.186
Constipation	0.0	-6.3	6.3	=0.199
Nausea/vomiting	2.8	-0.4	3.2	=0.056
Diarrhea	5.7	4.8	0.9	=0.147

*A positive change indicates an improvement in global health status and functional scales and a negative change indicates an improvement in symptom and single item scales.

†Based on a mixed analysis-of-covariance model with visit as a fixed effect, patient as a random effect and baseline as a covariate.

Relationship between FACIT-Fatigue quality of life and intravascular hemolysis

In order to determine if there was a treatment independent relationship between the FACIT-Fatigue quality of life instrument and intravascular hemolysis, an analysis was performed whereby the mean LDH level (through the 26 week study period) for each TRIUMPH patient was analyzed as a function of the patient's respective mean change in FACIT-Fatigue score from baseline (through the 26 week study period) (see Table 3). For this analysis, mean levels of LDH were divided into 4 groups that included: normal levels, 1-2 times the upper limit of normal (ULN), 2-10 times the upper limit of normal, and greater than 10 times the upper limit of normal. The analysis demonstrated that patients who maintained normal LDH levels throughout the study experienced significant improvements in fatigue when compared to patients who had incrementally higher levels of LDH throughout the study (p=0.0048). These data establish a clear link between increasing intravascular hemolysis as measured by LDH levels and decreased quality of life as measured by the FACIT-Fatigue quality of life instrument.

Table 3

Relationship between FACIT-Fatigue and Intravascular Hemolysis

Treatment Group	LDH Category	Change of FACIT from Baseline		p Value
		< 4	>= 4	
Combined	Normal	4 (30.77%)	9 (69.23%)	.0048
	1 - 2 X ULN	16 (61.54%)	10 (38.46%)	
	2 - 10 X ULN	18 (72.00%)	7 (28.00%)	
	> 10 X ULN	16 (80.00%)	4 (20.00%)	

Safety

There were no deaths in the study. Serious adverse events (SAEs) were reported for 13 patients, of which 4 occurred in the eculizumab-treated cohort and 9 were in the placebo-treated cohort (see Table 4). All patients recovered without sequelae.

The most common AEs reported for eculizumab-treated patients were headache, nasopharyngitis, back pain, and upper respiratory tract infection. Headache and back pain occurred more commonly in the eculizumab-treatment group compared with the placebo group. However, the increase in headaches was limited to the first 2 weeks of therapy and was mild to moderate. There were no statistically significant differences in incidents rates between treatment groups for any AEs reported.

One episode of thrombosis (Budd-Chiari) occurred in a placebo-treated patient. There were no thromboses in eculizumab-treated patients.

Only one patient showed a detectable level of anti-eculizumab antibodies in the eculizumab-treated cohort; this response was weak (did not titrate), occurred at only one time point and did not result in a disruption of complement blockade.

Table 4

Adverse Event Reporting

SERIOUS ADVERSE EVENTS*	Placebo Patients n (percent)	Eculizumab Patients n (percent)
Total	9 (20.5)	4 (9.3)
Eculizumab treatment emergent		
Exacerbation of PNH	3 (6.8)	1 (2.3)
Renal colic	0	1 (2.3)
Lumbar sacral disc prolapse	0	1 (2.3)
Alpha streptococcal bacteremia	0	1 (2.3)
Central line infection and UTI	1 (2.3)	0 (0)
Upper respiratory tract infection	1 (2.3)	0 (0)
Probable viral infection	1 (2.3)	0 (0)
Neutropenia	1 (2.3)	0 (0)
Cellulitis / folliculitis / neutropenia	1 (2.3)	0 (0)
Anemia and pyrexia	1 (2.3)	0 (0)
MOST FREQUENT ADVERSE EVENTS*†		
Headache§	12 (27.3)	19‡ (44.2)
Nasopharyngitis	8 (18.2)	10 (23.3)
Upper respiratory tract infection	10 (22.7)	6 (14)
Back pain	4 (9.1)	8 (18.6)
Nausea	5 (11.4)	7 (16.3)
Cough	4 (9.1)	5 (11.6)
Diarrhea	5 (11.4)	4 (9.3)
Arthralgia	5 (11.4)	3 (7.0)
Abdominal pain	5 (11.4)	2 (4.7)
Dizziness	5 (11.4)	2 (4.7)
Vomiting	5 (11.4)	2 (4.7)
Fatigue	1 (2.3)	5 (11.6)

Viral infection	5 (11.4)	1 (2.3)

*By preferred terms

†Occurring in 10% or more of patients

‡Sixteen of 19 patients experienced headache within 48 hours of infusion

§Following the first 2 weeks of dosing, 20.9% of eculizumab- and 22.7% of placebo-treated patients experienced headache

DISCUSSION

Chronic intravascular hemolysis with periods of acute exacerbation are the classical manifestations of PNH, frequently resulting in anemia, the need for transfusions to sustain hemoglobin levels, and deterioration in quality of life. In the phase III pivotal TRIUMPH study, we examined the effect of terminal complement inhibition with eculizumab on hemoglobin levels and transfusion requirements in patients with PNH. Forty-nine percent of patients treated with eculizumab over the 6-month period demonstrated stabilization of hemoglobin in the absence of transfusions compared to no patients in the placebo arm of the trial. Over 50% of eculizumab-treated patients were transfusion independent during the entire study compared to none in the placebo arm, and the overall mean transfusion rate was reduced by 73%. Moreover, even in patients who did not achieve transfusion independence, eculizumab treatment was associated with a 44% reduction in the rate of transfusion (data now shown).

Lactate dehydrogenase, a biochemical marker of hemolysis in PNH,[9] was immediately and consistently decreased in all eculizumab-treated patients, while patients in the placebo cohort continued to hemolyze with levels of LDH exceeding 5 times the upper limit of the normal range in all patients at the study end. Levels of LDH were reduced into the normal range in approximately one-third of eculizumab-treated patients, while the remainder stabilized at a level just above the upper limit of normal suggesting residual low level hemolysis in some patients. Levels of haptoglobin, a more sensitive marker of the presence of cell free hemoglobin in the circulation, were undetectable in most patients. Low level hemolysis in a subset of eculizumab-treated patients is possibly due to an inherent decrease in survival of these cells or C3b-mediated, extravascular clearance of PNH erythrocytes through the reticuloendothelial system.[17]

Samsung Bioepis Exhibit No. 1002
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Before eculizumab treatment, hemoglobin levels in study patients were artificially maintained by frequent transfusion. Therefore, stabilization of hemoglobin levels with a concomitant cessation of or reduction in transfusions represents a net increase in endogenous hemoglobin levels. Our data suggest that resolution of hemolysis with eculizumab results in a new steady state hemoglobin level determined by a balance between the extent of the underlying bone marrow dysfunction, the number of PNH erythrocytes that are preserved by eculizumab therapy and the new level (if any) of transfusion requirement.

Patients with PNH generally experience markedly impaired quality of life characterized by fatigue, anemia, thrombosis, and pulmonary hypertension as well as smooth muscle dystonia including abdominal pain, dysphagia, and erectile dysfunction.[9,10,18] These symptoms have been attributed to both excessive intravascular hemolysis and downstream scavenging of nitric oxide by cell free hemoglobin in plasma. The reduction of intravascular hemolysis in eculizumab-treated patients in the current study was associated with significant improvements in the fatigue component of quality of life relative to placebo-treated patients as assessed via the FACIT-Fatigue instrument. Further, eculizumab therapy was associated with a median increase of 6.4 points over baseline values established before treatment. It has previously been demonstrated that an increase of 3 or more points from baseline represents a clinically important difference in this quality of life instrument.[19] Patients who received eculizumab also experienced a significant improvement in most domains of the EORTC QLQ-30 relative to the placebo-treated cohort including global health status, physical functioning, emotional functioning, cognitive functioning, role functioning, social functioning, fatigue, pain, dyspnea, appetite loss, and insomnia. Improvement in the fatigue component of the EORTC QLQ-30 provides support for the improvement demonstrated in the FACIT-Fatigue instrument during eculizumab therapy. Importantly, these improvements in quality of life in the eculizumab-treated patients occurred despite similar levels of erythrocyte hemoglobin in the two treatment groups, further supporting the contribution of hemolysis per se, as opposed to anemia, in mediating the poor quality of life in PNH patients. Clinical assessment of additional life quality-related symptoms of PNH, including abdominal pain, dysphagia, and erectile dysfunction, have also been reported to improve during eculizumab therapy.[20]

Eculizumab was safe and well-tolerated. There were no deaths in the study and only a single thrombotic event which occurred in a placebo patient in a site (the hepatic veins) which is typical of the thrombosis in PNH. The relative brief duration of this study was not sufficient to address the relevant issue of a possible protection from thrombosis by terminal complement inhibition with eculizumab.

Adverse events were generally mild with headache occurring at increased frequency in the eculizumab-treated patients; however, this increased frequency did not persist following the first two doses of therapy. There were 4 SAEs in the eculizumab treatment group and 9 SAEs in the placebo group. There was no evidence of increased infection risk in eculizumab-treated patients during the study period. One eculizumab-treated patient showed a low level of anti-eculizumab antibodies at one time point during the study which did not persist and did not result in a disruption of complement blockade. There were no AEs associated with eculizumab withdrawal in the 2 eculizumab-treated patients who did not complete the trial. Additional safety assessments, as well as efficacy measures, are being examined in an ongoing multi-center, open-label Phase III safety trial of eculizumab (SHEPHERD) in approximately 95 patients with PNH.

Results from the current randomized, double-blind, placebo-controlled, global study show that terminal complement inhibition with eculizumab appears to be a safe and effective therapy for patients with the rare disorder PNH. Treatment with eculizumab reduced intravascular hemolysis, and stabilized hemoglobin levels despite a reduction of transfusions, to the point where most PNH patients were rendered transfusion independent. Substantial and clinically meaningful improvements in fatigue and other key quality of life parameters were also demonstrated. All of the 85 patients who completed the study elected to receive eculizumab in an open-label extension study and all currently remain on drug. The results of the TRIUMPH study indicate that terminal complement inhibition with eculizumab safely and effectively addresses an important consequence of the underlying genetic defect in PNH hematopoietic stem cells by providing a therapeutic replacement for the terminal complement inhibitor deficiency.

The present invention provides among other things treatment with an inhibitor of complement. Many variations of the invention will become apparent to those skilled in the art upon review of this specification. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

All publications and patents mentioned herein, including those items listed below, are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

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SEQUENCES

SEQ ID NO: 1 – Eculizumab V_H

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SEQ ID NO: 7 – Eculizumab CDRH3
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SEQ ID NO: 8 – Eculizumab CDRL1
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SEQ ID NO: 9 – Eculizumab CDRL2
GATNLAD

SEQ ID NO: 10 – Eculizumab CDRL3
QNVLNTPLT

WHAT IS CLAIMED IS:

1. A method of treating a patient suffering from paroxysmal nocturnal hemoglobinuria (PNH) comprising administering to the patient a pharmaceutical composition comprising an antibody that binds C5, wherein the antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

2. The method of claim 1, wherein the pharmaceutical composition is administered by intravenous infusion.

3. The method of claim 1, wherein the antibody is administered to the patient at a dosage level of between 5 mg per kg and 50 mg per kg per patient per treatment.

4. The method of claim 1, wherein the pharmaceutical composition is in a single unit dosage form.

5. The method of claim 4, wherein the single unit dosage form is a 300 mg single unit dosage form.

6. The method of claim 1, wherein the pharmaceutical composition comprises a 300 mg single-use formulation of 30 ml of a 10 mg/ml sterile, preservative free solution.

7. The method of claim 1, wherein the patient is anemic.

8. The method of claim 1, wherein the patient is dosed as follows: 600 mg of the antibody via intravenous infusion every 7±1 days for 4 doses; followed by 900 mg of the antibody via intravenous infusion 7±1 days later; followed by a maintenance dose of 900 mg of the antibody via intravenous infusion every 14±2 days.

9. The method of claim 1, wherein administration of the antibody results in an immediate and sustained decrease in mean levels of lactate dehydrogenase (LDH).

10. The method of claim 9, wherein the immediate decrease occurs within one week of administration of the antibody.

TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Eculizumab, a humanized monoclonal antibody against C5 that inhibits terminal complement activation, showed activity in a preliminary 12-week open-label trial in a small cohort of patients with paroxysmal nocturnal hemoglobinuria (PNH). The present study examined whether chronic eculizumab therapy could reduce intravascular hemolysis, stabilize hemoglobin levels, reduce transfusion requirements, and improve quality of life in a double-blind, randomized, placebo-controlled, multi-center global Phase III trial. It has been found that eculizumab stabilized hemoglobin levels, decreased the need for transfusions, and improved quality of life in PNH patients via reduced intravascular hemolysis. Chronic eculizumab treatment appears to be a safe and effective therapy for PNH.

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1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ile Phe Ser Asn Tyr
20 25 30

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

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Glu Tyr Thr Glu Asn Phe
50 55 60

Lys Asp Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

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

Ala Arg Tyr Phe Phe Gly Ser Ser Pro Asn Trp Tyr Phe Asp Val Trp
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Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
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<210> 2
<211> 448
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<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 2

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

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Glu Tyr Thr Glu Asn Phe
    50                  55                  60

Lys Asp Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65                  70                  75                  80

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

Ala Arg Tyr Phe Phe Gly Ser Ser Pro Asn Trp Tyr Phe Asp Val Trp
            100                 105                 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
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Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr
    130                 135                 140
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Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145                 150                 155                 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
                165                 170                 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
            180                 185                 190

Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp
        195                 200                 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys
    210                 215                 220

Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser
225                 230                 235                 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro
            260                 265                 270

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val
    290                 295                 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr
                325                 330                 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            340                 345                 350

Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
        355                 360                 365
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Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370                 375                 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390                 395                 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser
                405                 410                 415

Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            420                 425                 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
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<210> 3
<211> 131
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 3

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            20                  25                  30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gly Ala Ser
        35                  40                  45

Glu Asn Ile Tyr Gly Ala Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
    50                  55                  60

Ala Pro Lys Leu Leu Ile Tyr Gly Ala Thr Asn Leu Ala Asp Gly Val
65                  70                  75                  80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
                85                  90                  95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Asn
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Val Leu Asn Thr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
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Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gly Ala Ser
35 40 45

Glu Asn Ile Tyr Gly Ala Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
50 55 60

Ala Pro Lys Leu Leu Ile Tyr Gly Ala Thr Asn Leu Ala Asp Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Asn
100 105 110

Val Leu Asn Thr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

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

145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
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Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
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Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
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<210> 5
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peptide

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peptide

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peptide

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Gly Ala Thr Asn Leu Ala Asp
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peptide

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Gln Asn Val Leu Asn Thr Pro Leu Thr
1 5

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/260,888

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	10 minus 20 =	*			OR	x 80 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*				x 420 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1600

APPLICATION AS AMENDED - PART II

AMENDMENT A

	(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))	*	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	

AMENDMENT B

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

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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
15/260,888	09/09/2016	1677	1600	AXJ-114BUSCN3	10	1

CONFIRMATION NO. 2558

FILING RECEIPT

133485
Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

OC000000085897308

Date Mailed: 09/20/2016

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Leonard BELL, Woodbridge, CT;
Russell P. Rother, Oklahoma City, OK;

Applicant(s)

Alexion Pharmaceuticals, Inc., New Haven, CT;

Power of Attorney: The patent practitioners associated with Customer Number 133485

Domestic Priority data as claimed by applicant

This application is a CON of 15/148,839 05/06/2016
which is a CON of 13/426,973 03/22/2012
which is a CON of 12/225,040 05/13/2009 ABN
which is a 371 of PCT/US2007/006606 03/15/2007

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

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

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

If Required, Foreign Filing License Granted: 09/19/2016

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/260,888**

Projected Publication Date: 12/29/2016

Non-Publication Request: No

Early Publication Request: No

Title

TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Preliminary Class

435

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

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

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

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

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

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Sep 20, 2016 05:23:25 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

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Application	Document	Mailroom Date	Attorney Docket No.
15260888	APP.FILE.REC	09/20/2016	AXJ-114BUSCN3

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

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Thank you for prompt attention to this notice,

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PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3	2558

133485 7590 09/29/2016

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
09/29/2016	ELECTRONIC

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

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

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

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

UNITED STATES PATENT AND TRADEMARK OFFICE

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

Doc Code:
TRACK1.GRANT

Decision Granting Request for Prioritized Examination (Track I or After RCE)	Application No.:15/260,888

1. THE REQUEST FILED September 9, 2016 IS **GRANTED.**

The above-identified application has met the requirements for prioritized examination
A. ☒ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination.** The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;
B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims**, or a multiple dependent claim;
C. filing a **request for continued examination**;
D. filing a notice of appeal;
E. filing a request for suspension of action;
F. mailing of a notice of allowance;
G. mailing of a final Office action;
H. completion of examination as defined in 37 CFR 41.102; or
I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to Kimberly Inabinet at 571-272-4618.

/Kimberly Inabinet/ Paralegal Specialist, Office of Petitions

Office of Petitions: Routing Sheet

Application No. 15/260,888

This application is being forwarded to your office for further processing. A decision has been rendered on a petition filed in this application, as indicated below. For details of this decision, please see the document PET.OP.DEC filed on the same date as this document.

[X] **GRANTED**

[ ] **DISMISSED**

[ ] **DENIED**

Office of Petitions: Decision Count Sheet

Mailing Month

Application No. 15260888

15260888

For US serial numbers: enter number only, no slashes or commas. Ex: 10123456

For PCT: enter "51+single digit of year of filing+last 5 numbers", Ex. for PCT/US05/12345, enter 51512345

Deciding Official: Kimberly Inabinet

Count (1) - Palm Credit 15/260,888

Decision: GRANT

FINANCE WORK NEEDED

☐ Select Check Box for YES

GRANT

Decision Type: 643 - Track One request

643

Notes:

Count (2)

Decision: n/a

FINANCE WORK NEEDED

☐ Select Check Box for YES

Decision Type: NONE

Notes:

Count (3)

Decision: n/a

FINANCE WORK NEEDED

☐ Select Check Box for YES

Decision Type: NONE

Notes:

Initials of Approving Official (if required)

If more than 3 decisions, attach 2nd count sheet & mark this box ☐

Printed on: *9/26/2016*

Office of Petitions Internal Document - Ver. 5.0

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Sep 29, 2016 05:26:37 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

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Application	Document	Mailroom Date	Attorney Docket No.
15260888	PET.OP.DEC	09/29/2016	AXJ-114BUSCN3
	TRACK1.GRANT	09/29/2016	AXJ-114BUSCN3

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If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

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PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: October 21, 2016
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, /

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888 Confirmation No.: 2558

Filed: September 9, 2016 Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT (IDS)

Dear Sir:

In compliance with 37 C.F.R. § 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the documents listed on the attached PTO/SB/08. It is respectfully requested that the documents listed on the PTO/SB/08 be expressly considered by the Examiner during the prosecution of this application, and that the documents be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

The present application is a continuation of U.S. Application No. 15/148,839, filed May 6, 2016 (Atty. Docket No. AXJ-114BUSCN2). For the Examiner's convenience in reviewing this continuation application, Applicant submits a consolidated PTO Form SB/08, listing all references cited during the prosecution of the parent applications [U.S. Application No. 15/148,839, filed May 6, 2016 (Atty. Docket No. AXJ-114BUSCN2), U.S. Application No. 13/426,973, filed March 22, 2012 (Atty. Docket No. AXJ-114BUSCN), and U.S. Application No. 12/225,040, filed May 13, 2009 (Atty. Docket No. AXJ-0114BUS)].

Copies of the references previously cited by or submitted to the Office in the parent applications are not enclosed, but will be provided upon request, in accordance with 37 CFR §1.98(d). In addition, Applicant submits herewith copies of foreign patents and non-patent literature references, but has not included copies of U.S. patents and U.S. patent applications, in accordance with 37 C.F.R. § 1.98(a)(2)(i)-(iv).

Additionally, Applicant calls to the attention of the Examiner the following Applications and Office Actions cited therein:

Applications				
Examiner's Initials	*Serial No.*	*Filing Date*	*First Named Inventor*	*Docket No.*
	15/284,015	10-03-2016	Leonard Bell	AXJ-114BUSCN4
	15/148,839	05-06-2016	Leonard Bell	AXJ-114BUSCN2
	13/426,973	03-22-2012	Leonard Bell	AXJ-114BUSCN
	12/225,040	05-13-2009	Leonard Bell	AXJ-114BUS
	12/621,792	11-19-2009	Leonard Bell	AXJ-114DV
	10/771,552	02-03-2004	Leonard Bell	AXJ-114RCE
	11/050,543	02-03-2005	Leonard Bell	AXJ-114CPRCE
	11/595,118	11-08-2006	Leonard Bell	AXJ-114CP2
	12/902,617	10-12-2010	Leonard Bell	AXJ-114CPCN
	13/335,518	12-22-2011	Leonard Bell	AXJ-114CPCN2RCE

Office Actions (copies enclosed)			
Examiner's Initials	Serial No.	Date Mailed from USPTO	Examiner
	15/148,839	08-16-2016	J. Rogers
	13/426,973	01-27-2014	J. Rogers
	13/426,973	05-31-2013	J. Rogers
	13/426,973	08-20-2012	J. Rogers
	12/225,040	09-22-2011	J. Rogers
	12/225,040	06-01-2011	J. Rogers
	12/225,040	04-11-2011	J. Rogers
	12/621,792	09-30-2010	P. Gambel
	10/771,552	12-22-2008	F. Vandervegt
	10/771,552	03-21-2008	F. Vandervegt
	10/771,552	08-10-2007	F. Vandervegt
	10/771,552	02-26-2007	F. Vandervegt
	10/771,552	09-26-2006	F. Vandervegt

	11/050,543	07-09-2010	P. Gambel
	11/050,543	04-02-2008	F. Vandervegt
	11/050,543	10-10-2007	F. Vandervegt
	11/050,543	06-11-2007	F. Vandervegt
	11/595,118	01-21-2011	P. Gambel
	11/595,118	10-05-2010	P. Gambel
	11/595,118	07-07-2010	P. Gambel
	11/595,118	04-03-2009	F. Vandervegt
	12/902,617	06-23-2011	P. Gambel
	12/902,617	04-04-2011	P. Gambel
	13/335,518	08-22-2016	P. Gambel
	13/335,518	09-12-2014	P. Gambel
	13/335,518	02-06-2014	P. Gambel
	13/335,518	11-07-2013	P. Gambel

The Examiner is requested to review the file histories of these applications, including cited references, Office Actions, Responses, etc., and is asked to contact Applicant's Attorney if the Examiner would like the Applicant to supply copies of any or all of the information included in any of these applications. For any of these applications, if Applicant's Attorney is not contacted by the Examiner with such a request, then it will be concluded that the Examiner has reviewed or will review the file content of these applications.

Applicant respectfully requests that the Examiner initial the blank columns next to the cited Applications and Office Actions, to indicate that the information has been considered by the Examiner. Alternatively, Applicant requests that the Examiner insert the phrase, "All references considered except where lined through," on each page of the Information Disclosure Statement, along with the Examiner's initials.

The filing of this Information Disclosure Statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any documents herein be construed *per se* as a representation that such document is prior art. Moreover, Applicant understands the Examiner will make an independent evaluation of the cited documents.

This Information Disclosure Statement is filed within three months of the U.S. filing date (37 C.F.R. § 1.97(b)(1)). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3.

Dated: October 21, 2016

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, /
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

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

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99**)		
	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

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	2	HILL et al., Protection of erythrocytes from human complement-mediated lysis by membranetargeted; recombinant soluble CD59: a new approach to PNH therapy, Blood 107(5):2131-; 2137 (2006);	
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Examiner Signature		Date Considered	

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	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

CERTIFICATION STATEMENT

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

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

OR

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

See attached certification statement.

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

A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Jill Gorny Sloper/	Date (YYYY-MM-DD)	2016-10-21
Name/Print	Jill Gorny Sloper, Esq.	Registration Number	60,760

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

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	Attorney Docket Number	AXJ-114BUSCN3

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	1	THOMAS et al., Inhibition of complement activity by humanized anti-C5 antibody and single; chain Fv, Mol. Imm. 33 (17-18):1389-1401 (1996);	
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Examiner Signature		Date Considered	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

CERTIFICATION STATEMENT

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

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

OR

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

See attached certification statement.

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

A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Jill Gorny Sloper/	Date (YYYY-MM-DD)	2016-10-21
Name/Print	Jill Gorny Sloper, Esq.	Registration Number	60,760

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

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

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

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

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Electronic Acknowledgement Receipt	
EFS ID:	27283505
Application Number:	15260888
International Application Number:	
Confirmation Number:	2558
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Anita Costa
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3
Receipt Date:	21-OCT-2016
Filing Date:	09-SEP-2016
Time Stamp:	17:28:58
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Other Reference-Patent/App/Search documents	10771552.pdf	1115237 e3a89e9c3877787ada055bda3925f0f4d1f40a8a	no	26

Warnings:

Information:					
2	Other Reference-Patent/App/Search documents	11050543.pdf	1248587 4d4e8fa198834e1f4bdfc2e78ed07b6a89c5b493	no	27
Warnings:					
Information:					
3	Other Reference-Patent/App/Search documents	11595118.pdf	1336578 138e6ad08e8059db9828fc3b209fc4fa6c083838	no	28
Warnings:					
Information:					
4	Other Reference-Patent/App/Search documents	12225040.pdf	794072 fe79d9838d9718506cb151f86188c00c803c7a1c	no	17
Warnings:					
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5	Other Reference-Patent/App/Search documents	12621792.pdf	831144 522897dd58c3e8bb9ecc869ef4e18034ae8d8dba	no	14
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6	Other Reference-Patent/App/Search documents	12902617.pdf	988774 ca5082bb6aa229b368848c9f9af2692cc5d427e8	no	19
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7	Other Reference-Patent/App/Search documents	13426973.pdf	1459620 f9f777145b54b47f770f50c521a65ebb31250c36	no	27
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8	Other Reference-Patent/App/Search documents	13335518.pdf	2968685 89b0a15b2d31c70047812f4fd9f75fc0a264e9a1	no	54
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9	Other Reference-Patent/App/Search documents	15148839.pdf	273044 d3d63c344fcfd7770cf118d36bed14cffb8b8c17	no	6
Warnings:					
Information:					
10	Non Patent Literature	Adis_International_Limited.pdf	28755 214709e7da5c98817765713ed1a02cc69b477328	no	2
Warnings:					
Information:					
11	Non Patent Literature	Alexion_completes_enrollment_in_Ph_II_kidney_disease_trial.pdf	29992 693f575b2bd0395315cbe9dd1ae0f9924069058e	no	2
Warnings:					
Information:					
12	Non Patent Literature	Alexion_Completes_Enrollment_in_Phase_II_Membranous_02.pdf	38622 258a054bd64de1368286fde21a36fb4e993832fd	no	3
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Information:					
13	Non Patent Literature	Alexion_issued_C5_complement_inhibitor_patent_for_inflammatory_diseases.pdf	22215 b61a48ce2df53309fc1c1f4552b50bbf4e85c82d	no	1
Warnings:					
Information:					
14	Non Patent Literature	Alexion_Issued_Key_C5_Complement_Inhibitor_Patent_for_Inflammatory_Diseases_02.pdf	35493 1e864dd9d7439d22029401f54c28b562d529730a	no	3
Warnings:					
Information:					
15	Non Patent Literature	ALEXION_OFFERS_EARLY_MIXEDPHASE_III_DATA_FOR_HEART_DRUG.pdf	36354 18abf3135d8d4fc7785c526902b613daffada800	no	3
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Information:					

16	Non Patent Literature	Alexion_Pharmaceuticals_Inc_Kidney_Disease.pdf	31473 25a2f091c6dc9a1961a3deb99c246cb94f03f616	no	2
Warnings:					
Information:					
17	Non Patent Literature	Alexion_Pharmaceuticals_IncKidney_Disease__14.pdf	31235 8ceff4577262b02a0794ed283fce22a1e0f5ecb6	no	2
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Information:					
18	Non Patent Literature	Alexion_Pharmaceuticals_Reports_Second_Quarter_and_First_Half_Results_03.pdf	49539 68a13e5a4b9f36090d97e9524f11a011f522d98a	no	8
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Information:					
19	Non Patent Literature	Alexion_Pharmaceuticals_Reports_Second_Quarter_and_First_Half_Results_-_Quarter_Highlight.pdf	51469 734e29a01d86e56986ee878728a95eb2ece396e5	no	6
Warnings:					
Information:					
20	Non Patent Literature	Alexion_Pharmaceuticals_Inc_Company_reports_second_quarter_first-_half_results.pdf	32779 8297011ea7d8d63a3e7c63baa3ef8e7fdfdd59ba	no	2
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Information:					
21	Non Patent Literature	Brekke.pdf	1280489 f1fc73123aee0451d2d98cd90080d690262b2ca8	no	11
Warnings:					
Information:					
22	Non Patent Literature	CELLA_-OncologyWilliston_Park_1111A_232-2351997__11_.pdf	29250 9f473af7971d569d2bc8eea2cfe53172663832d8	no	1
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23	Non Patent Literature	eculizumab_Alexion_clinical_data_phase_III_PNH.pdf	28006 824d34e4bed4e31451c73442e4f95ce805138987	no	2
Warnings:					
Information:					
24	Non Patent Literature	eculizumab_Alexion_clinical_data_PNH_R__D_Focus_Drug_News_.pdf	28334 bc64556124f25c7738b7de05e7c6d7fb109f3ad0	no	2
Warnings:					
Information:					
25	Non Patent Literature	eculizumab_Alexion_completes_enrollment_02.pdf	29118 43630129828fb68271fc533c261ba5a927875132	no	2
Warnings:					
Information:					
26	Non Patent Literature	eculizumab_Alexion_completes_enrollment_for_phase_III_PNH_trial.pdf	30519 400a43524514a4b8d782eab2e5278f72acfed461	no	2
Warnings:					
Information:					
27	Non Patent Literature	eculizumab_Alexion_initiates_second_phase_III_study.pdf	31235 7512bd81de01aab49c1c9357b2c22aa02b6a34aa	no	2
Warnings:					
Information:					
28	Non Patent Literature	eculizumab_Alexion_phase_change_III_USA_Europe_Canada_Australia_PNH.pdf	32777 82916339def85f3c19629eb208a578973f6725bf	no	2
Warnings:					
Information:					
29	Non Patent Literature	eculizumab_Alexion_plans_phase_III_trials_paroxysmal_nocturnal_hemoglobinuria.pdf	30749 e4885c3058b052f2a028448f864bce1f5758345a	no	2
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30	Non Patent Literature	eculizumab_Alexion_Priority_Review_USA_PNH.pdf	27857 4cf10a374b77c33fd430739a36de9c6ae540d91b	no	2
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Information:					
31	Non Patent Literature	ESPICOM_Pharmaceutical_and_Medical_Device_News.pdf	28013 ca9d416b3724c3e3bee5d6ff237dbfd44a7ac7ec	no	2
Warnings:					
Information:					
32	Non Patent Literature	Fung.pdf	224992 a3fa008ad36f05c21be2718c500073d880bc6598	no	10
Warnings:					
Information:					
33	Non Patent Literature	Martin.pdf	587544 a374e9b2f102cd915ea8df9393f6652a603766d8	no	11
Warnings:					
Information:					
34	Non Patent Literature	MOLLNES.pdf	630714 12982d854797ca3f7e98faaf081c59ef2f63933a	no	15
Warnings:					
Information:					
35	Non Patent Literature	Osbourn_03.pdf	1891854 65342efe55c9b71a8f19ebe9c5311fed72ce36d1	no	10
Warnings:					
Information:					
36	Non Patent Literature	Other_News_To_Note.pdf	42576 1ff8944b729b75b56cee194e64ac04b2a4b0ee0c	no	5
Warnings:					
Information:					

37	Non Patent Literature	Pierangeli.pdf	292873 2030817c44f3a9ae9e447d534e3fecd50a76dec3	no	9
Warnings:					
Information:					
38	Non Patent Literature	R__D_Focus_Drug_News_Aug_28_2006.pdf	28894 46fe16eba2b4560cbc51c46f8f87e5c3c5cbd4f2	no	2
Warnings:					
Information:					
39	Non Patent Literature	Ricklin.pdf	1224810 4720fe3765d191716612b3523455200f3c661257	no	11
Warnings:					
Information:					
40	Foreign Reference	WO2005110481A2.pdf	3921591 2ee498bfad15b6303219e24a66f5e92a577661bf	no	73
Warnings:					
Information:					
41	Foreign Reference	1021111601_WO2004029207A2.pdf	9710349 7aeb2e2aa0c56fb560a8f2733e1def2e81bc3bfb	no	192
Warnings:					
Information:					
42	Foreign Reference	1021111722_WO2005074607A2.pdf	3444312 eacb18466e2d92227241a717baab3dcc0692fd41	no	83
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43	Information Disclosure Statement (IDS) Form (SB08)	IDSTRANS.pdf	17797 9233100d9a0921721d788c1ebe3c7f2388c43320	no	4
Warnings:					
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44	Information Disclosure Statement (IDS) Form (SB08)	SB01.pdf	1040190 441506529da73636453ab928c2f906971488cc24	no	11
Warnings:					
Information:					
45	Information Disclosure Statement (IDS) Form (SB08)	SB02.pdf	1039627 aa4631914b60be8b5a30a102bcb7c7f45bd48469	no	8
Warnings:					
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A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.					
46	Information Disclosure Statement (IDS) Form (SB08)	SB03.pdf	1035264 74e5386b079483e3c1a4d714753daf1f613e6cdd	no	4
Warnings:					
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A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.					
Total Files Size (in bytes):			38113401		

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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.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/771,552	02/03/2004	Leonard Bell	ALXN-PO1-114	6183

28120 7590 12/22/2008

ROPES & GRAY LLP
PATENT DOCKETING 39/41
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624

EXAMINER	
VANDERVEGT, FRANCOIS P	
ART UNIT	PAPER NUMBER
1644	

MAIL DATE	DELIVERY MODE
12/22/2008	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)	
	10/771,552	BELL ET AL.	
	Examiner	Art Unit	
	F. Pierre VanderVegt	1644	

-- 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 *23 June 2008*.

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

3)☐ 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

4)☒ Claim(s) *109-120* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *109-120* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *20080623*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The instant application, filed on February 3, 2004, does not claim priority to any earlier application.

Claims 1-108 and 121-171 have been canceled.

Claims 109-120 are currently pending and are the subject of examination in the present Office Action.

In view of Applicant's remarks filed June 23, 2008 the following ground of rejection is maintained.

Claim Rejections - 35 USC § 103

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.

1. Claims 109-120 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Alexion press release dated January 6, 2003 (CG on form PTO-1449 filed 5/10/2007, of record) in view of Collard et al (Arterioscler Thromb Vasc Biol. [1999]19(11):2623-2629; U on form PTO-892, newly cited).

It was previously stated: "The Alexion press release teaches the use of the anti-C5 antibody compound eculizumab (h5G1.1-scFv) for the treatment of subjects with the hemolytic disease paroxysmal nocturnal hemoglobinuria (see entire document). It is noted that h5G1.1-scFv is the same compound recited in claim 111. The Alexion press release is silent about the treatment of NO deficiency in paroxysmal nocturnal hemoglobinuria. However, because the compound used to treat paroxysmal nocturnal hemoglobinuria in the Alexion press release and the instantly claimed compound to treat NO deficiency in paroxysmal nocturnal hemoglobinuria are the same, the treatment of NO deficiency in paroxysmal nocturnal hemoglobinuria would be an inherent property of the anti-C5 antibody compound eculizumab (h5G1.1-scFv). The prior art teaching anticipates the claimed invention.

The Alexion press release does not specifically link the hemolytic disease with NO deficiency or the effect of h5G1.1-scFv on NO levels.

Collard teaches the treatment of hypoxic HUVECs with h5G1.1-scFv (see entire document). Collard teaches that terminal complement component C5b-9 deposition results in a functional loss of NO-dependent relaxation (page 2625 in particular), increases VCAM-1 expression and decreases cGMP levels (page 2623 and page 2625 in particular). Collard teaches that decreased cGMP levels may compromise vascular blood flow because of decreased endothelium-dependent relaxation and increased adhesion of

neutrophils to the endothelium (page 2623 in particular). Collard teaches that h5G1.1-scFv treatment of the HUVECS attenuates C5b-9 deposition and preserves acetylcholine induced increases in cGMP after hypoxia/reoxygenation (page 2625 in particular).

It would have been prima facie obvious to a person having ordinary skill in the art at the time the invention was made to use the h5G1.1-scFv antibody taught by the Alexion press release for the treatment of NO deficiency in a subject. One would have been motivated to treat NO deficiency with h5G1.1-scFv with a reasonable expectation of success by the showing of Alexion that h5G1.1-scFv treatment of PNH relieved hemolysis and the teachings of Collard that C5b-9 deposition during reoxygenation after a hypoxic event inhibited NO-mediated cGMP expression, which adversely affects vascular blood flow and attracts damaging neutrophils to the endothelial surface. Collard teaches that treatment with h5G1.1-scFv inhibits this C5b-9 deposition and attenuates cGMP loss.

Claims 112-114 are included because, while the references are silent about the proportion of type III red blood cells, silence about a particular property does not necessarily constitute absence of that property. Also, claims 115-117 are included because, while the references are silent about the platelet counts in a subject, silence about a particular property does not necessarily constitute absence of that property. Furthermore claims 118-120 are included because, while the references are silent about the reticulocyte counts in a subject, silence about a particular property does not necessarily constitute absence of that property. The office does not have the facilities and resources to provide the factual evidence needed in order to establish that there is a difference between the materials, i.e., that the claims are directed to new materials and that such a difference would have been considered unexpected by one of ordinary skill in the art, that is, the claimed subject matter, if new, is unobvious. In the absence of evidence to the contrary, the burden is on the Applicant to prove that the claimed materials are different from those taught by the prior art and to establish patentable differences. See In re Best 562F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray 10 USPQ 2d 1922 (PTO Bd. Pat. App. & Int. 1989)."

Applicant's arguments filed June 23, 2008 have been fully considered but they are not persuasive. Applicant argues that the instantly claimed invention cannot be considered obvious over the teachings of the Alexion press release in view of Collard et al because the "Alexion press release was specific to treating PNH patients" and "does not disclose that all patient populations could be treated for NO deficiency with compounds which bind to one or more complement components." Applicant further argues that the artisan would not expect that anti-C5 antibodies would relieve a functional loss of NO-dependent relaxation under all circumstances based on the teaching of Collard et al." Applicant argues therefore that because the scope of the prior art teaching is not as broad as the scope of what is recited in the instant claims, the instantly claimed invention cannot be obvious over the cited references. Applicant's arguments are without merit. In the instant case, the claims are drawn to the genus of conditions involving NO deficiency, while the teachings of the combined references are drawn to a species of NO deficiency in PNH. The prior art does not need to teach the entire genus of the claimed invention. A teaching of a species encompassed by that genus is sufficient to render the genus obvious.

Conclusion

2. No claim is allowed.

3. **THIS ACTION IS MADE FINAL.** Applicant is 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.

4. Any inquiry concerning this communication or earlier communications from the examiner should be directed to F. Pierre VanderVegt whose telephone number is (571)272-0852. The examiner can normally be reached on M-Th 6:30-4:00 and Alternate Fridays 6:30-3:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Eileen O'Hara can be reached on (571) 272-0878. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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F. Pierre VanderVegt, Ph.D. /PV/
Patent Examiner
March 17, 2008

/Eileen B. O'Hara/
Supervisory Patent Examiner
Art Unit 1644

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/771,552	02/03/2004	Leonard Bell	ALXN-PO1-114	6183

28120 7590 03/21/2008

ROPES & GRAY LLP
PATENT DOCKETING 39/41
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624

EXAMINER	
VANDERVEGT, FRANCOIS P	
ART UNIT	PAPER NUMBER
1644	

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03/21/2008	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)	
	10/771,552	BELL ET AL.	
	Examiner	Art Unit	
	F. Pierre VanderVegt	1644	

-- 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.

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- 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 *10 January 2008*.

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

3)☐ 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

4)☒ Claim(s) *109-120* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *109-120* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *20080110*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The instant application, filed on February 3, 2004, does not claim priority to any earlier application.

Claims 1-108 and 121-171 have been canceled.

Claims 109-120 are currently pending and are the subject of examination in the present Office Action.

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on January 10, 2008 has been entered.

In view of Applicant's amendment filed January 10, 2008 no outstanding ground of rejection is maintained.

Claim Rejections - 35 USC § 103

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.

2. Claims 109-120 are rejected under 35 U.S.C. 103(a) as being unpatentable over Alexion press release dated January 6, 2003 (CG on form PTO-1449 filed 5/10/2007, of record) in view of Collard et al (Arterioscler Thromb Vasc Biol. [1999]19(11):2623-2629; U on form PTO-892, newly cited).

The Alexion press release teaches the use of the anti-C5 antibody compound eculizumab (h5G1.1-scFv) for the treatment of subjects with the hemolytic disease paroxysmal nocturnal hemoglobinuria (see entire document). It is noted that h5G1.1-scFv is the same compound recited in claim 111. The Alexion press release is silent about the treatment of NO deficiency in paroxysmal

nocturnal hemoglobinuria. However, because the compound used to treat paroxysmal nocturnal hemoglobinuria in the Alexion press release and the instantly claimed compound to treat NO deficiency in paroxysmal nocturnal hemoglobinuria are the same, the treatment of NO deficiency in paroxysmal nocturnal hemoglobinuria would be an inherent property of the anti-C5 antibody compound eculizumab (h5G1.1-scFv). The prior art teaching anticipates the claimed invention.

The Alexion press release does not specifically link the hemolytic disease with NO deficiency or the effect of h5G1.1-scFv on NO levels.

Collard teaches the treatment of hypoxic HUVECs with h5G1.1-scFv (see entire document). Collard teaches that terminal complement component C5b-9 deposition results in a functional loss of NO-dependent relaxation (page 2625 in particular), increases VCAM-1 expression and decreases cGMP levels (page 2623 and page 2625 in particular). Collard teaches that decreased cGMP levels may compromise vascular blood flow because of decreased endothelium-dependent relaxation and increased adhesion of neutrophils to the endothelium (page 2623 in particular). Collard teaches that h5G1.1-scFv treatment of the HUVECS attenuates C5b-9 deposition and preserves acetylcholine induced increases in cGMP after hypoxia/reoxygenation (page 2625 in particular).

It would have been prima facie obvious to a person having ordinary skill in the art at the time the invention was made to use the h5G1.1-scFv antibody taught by the Alexion press release for the treatment of NO deficiency in a subject. One would have been motivated to treat NO deficiency with h5G1.1-scFv with a reasonable expectation of success by the showing of Alexion that h5G1.1-scFv treatment of PNH relieved hemolysis and the teachings of Collard that C5b-9 deposition during reoxygenation after a hypoxic event inhibited NO-mediated cGMP expression, which adversely affects vascular blood flow and attracts damaging neutrophils to the endothelial surface. Collard teaches that treatment with h5G1.1-scFv inhibits this C5b-9 deposition and attenuates cGMP loss.

Claims 112-114 are included because, while the references are silent about the proportion of type III red blood cells, silence about a particular property does not necessarily constitute absence of that property. Also, claims 115-117 are included because, while the references are silent about the platelet counts in a subject, silence about a particular property does not necessarily constitute absence of that property. Furthermore claims 118-120 are included because, while the references are silent about the reticulocyte counts in a subject, silence about a particular property does not necessarily constitute absence of that property. The office does not have the facilities and resources to provide the factual evidence needed in order to establish that there is a difference between the materials, i.e., that the claims are directed to new materials and that such a difference would have been considered unexpected by one of

ordinary skill in the art, that is, the claimed subject matter, if new, is unobvious. In the absence of evidence to the contrary, the burden is on the Applicant to prove that the claimed materials are different from those taught by the prior art and to establish patentable differences. See In re Best 562F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray 10 USPQ 2d 1922 (PTO Bd. Pat. App. & Int. 1989).

Conclusion

3. No claim is allowed.

4. Any inquiry concerning this communication or earlier communications from the examiner should be directed to F. Pierre VanderVegt whose telephone number is (571)272-0852. The examiner can normally be reached on M-Th 6:30-4:00 and Alternate Fridays 6:30-3:00.

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F. Pierre VanderVegt, Ph.D. /PV/
Patent Examiner
March 17, 2008

/David A Saunders/
Primary Examiner, Art Unit 1644

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/771,552	02/03/2004	Leonard Bell	ALXN-PO1-114	6183

28120 7590 08/10/2007

FISH & NEAVE IP GROUP
ROPES & GRAY LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624

EXAMINER	
VANDERVEGT, FRANCOIS P	

ART UNIT	PAPER NUMBER
1644	

MAIL DATE	DELIVERY MODE
08/10/2007	PAPER

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

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Office Action Summary	Application No.	Applicant(s)	
	10/771,552	BELL ET AL.	
	Examiner	Art Unit	
	F. Pierre VanderVegt	1644	

-- 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 *10 May 2007*.

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

3)☐ 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

4)☒ Claim(s) *1-171* is/are pending in the application.

4a) Of the above claim(s) *1-108 and 121-171* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *109-120* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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)).

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4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The instant application, filed on February 3, 2004, does not claim priority to any earlier application.

Claims 1-171 are currently pending.

Election/Restrictions

1. Applicant's election without traverse of Group VI, claims 109-120, in the reply filed on November 24, 2006 is acknowledged.

Claims 1-108 and 121-171 stand withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on November 245, 2006.

Accordingly, claims 109-120 are the subject of examination in the present Office Action.

2. This application contains claims drawn to an invention nonelected without traverse in the reply filed on November 24, 2006. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

In view of Applicant's amendment filed May 10, 2007 no outstanding grounds of rejection are maintained.

The following new ground of rejection has been necessitated by Applicant's amendment.

Claim Rejections - 35 USC § 102

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

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

3. Claims 109-120 are rejected under 35 U.S.C. 102(b) as being anticipated by Alexion press release dated January 6, 2003 (CG on form PTO-1449 filed 5/10/2007).

The Alexion press release teaches the use of the anti-C5 antibody compound eculizumab (h5G1.1-scFv) for the treatment of subjects with paroxysmal nocturnal hemoglobinuria (see entire

document). It is noted that h5G1.1-scFv is the same compound recited in claim 111. The Alexion press release is silent about the treatment of NO deficiency in paroxysmal nocturnal hemoglobinuria. However, because the compound used to treat paroxysmal nocturnal hemoglobinuria in the Alexion press release and the instantly claimed compound to treat NO deficiency in paroxysmal nocturnal hemoglobinuria are the same, the treatment of NO deficiency in paroxysmal nocturnal hemoglobinuria would be an inherent property of the anti-C5 antibody compound eculizumab (h5G1.1-scFv). The prior art teaching anticipates the claimed invention.

Claims 112-114 are included because, while the references are silent about the proportion of type III red blood cells, silence about a particular property does not necessarily constitute absence of that property. Also, claims 115-117 are included because, while the references are silent about the platelet counts in a subject, silence about a particular property does not necessarily constitute absence of that property. Furthermore claims 118-120 are included because, while the references are silent about the reticulocyte counts in a subject, silence about a particular property does not necessarily constitute absence of that property. The office does not have the facilities and resources to provide the factual evidence needed in order to establish that there is a difference between the materials, i.e., that the claims are directed to new materials and that such a difference would have been considered unexpected by one of ordinary skill in the art, that is, the claimed subject matter, if new, is unobvious. In the absence of evidence to the contrary, the burden is on the Applicant to prove that the claimed materials are different from those taught by the prior art and to establish patentable differences. See In re Best 562F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray 10 USPQ 2d 1922 (PTO Bd. Pat. App. & Int. 1989).

Conclusion

4. No claim is allowed.

5. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is 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 date of this final action.

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If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on (571) 272-0841. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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F. Pierre VanderVegt, Ph.D. /PV/
Patent Examiner
August 6, 2007

DAVID A. SAUNDERS
PRIMARY EXAMINER

UNITED STATES PATENT AND TRADEMARK OFFICE

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28120 7590 02/26/2007

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EXAMINER	
VANDERVEGT, FRANCOIS P	
ART UNIT	PAPER NUMBER
1644	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	02/26/2007	PAPER

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If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

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Status

1)☒ Responsive to communication(s) filed on *24 November 2006*.

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

3)☐ 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

4)☒ Claim(s) *1-171* is/are pending in the application.

4a) Of the above claim(s) *1-108 and 121-171* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *109-120* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

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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:

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4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The instant application, filed on February 3, 2004, does not claim priority to any earlier application.

Claims 1-171 are currently pending.

Election/Restrictions

1. Applicant's election without traverse of Group VI, claims 109-120, in the reply filed on November 24, 2006 is acknowledged.

2. Claims 1-108 and 121-171 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on November 245, 2006.

Accordingly, claims 109-120 are the subject of examination in the present Office Action..

Claim Rejections - 35 USC § 103

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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

3. Claims 109-120 are rejected under 35 U.S.C. 103(a) as being unpatentable over Vakeva et al (Circulation [1998] 97:2259-2267; U on form PTO-892) in view of Reiter et al (Nature Medicine [2002] 8(12):1383-1389; V on form PTO-892) and Fitch et al (Circulation [1999] 100:2499-2506; CA cited on form PTO-1449 filed 08/24/2006), as evidenced by Claque et al (Biomed. Instrum. Technol. [1995] 29(5):419-424, Abstract only; W on form PTO-892).

The claims are drawn to the treatment of nitric oxide deficiency in a subject having a hemolytic disease. Vakeva teaches that subjects who undergo prolonged periods of myocardial ischemia experience tissue injury and cell death (Abstract and page 2259, column 1 in particular). Vakeva relates the damage to activated terminal complement components (Abstract in particular). Vakeva teaches that treatment with an anti-C5 antibody inhibits apoptosis and necrosis and blocks greater than 80% of serum hemolytic activity (Abstract, Figure 8, section bridging pages 2265-2266 and paragraph bridging 2264-2265 in particular).

Reiter teaches that cell-free hemoglobin in sickle cell disease limits the availability of nitric oxide (NO)(Abstract in particular). Reiter teaches that free hemoglobin in the serum binds to NO 1,000 more rapidly than hemoglobin sequestered in erythrocytes. Reiter teaches that NO can only reach levels needed for activating guanylyl cyclase in smooth muscle to cause vasodilation only when hemoglobin is sequestered in erythrocytes (page 1383, column 1 in particular). Reiter further teaches that this condition in sickle cell is similar to that seen in clinical diseases such as hemolysis during cardiopulmonary bypass procedures (page 1387, column 2 in particular).

Fitch teaches that coronary bypass surgery (CBP) elicits a systemic inflammatory response (page 2499, column 1 in particular). Fitch teaches a method of administering of a humanized single chain monoclonal antibody directed to human complement component C5 (h5G1.1-scFv) to subjects undergoing CBP. The h5G1.1-scFv antibody is a complement inhibitor that binds to complement component C5 and prevents the cleavage of C5 into C5a and C5b (Fitch page 2500, column 1 in particular). Fitch teaches that h5G1.1-scFv treatment inhibited pathological complement activation, had a pronounced anti-inflammatory effect, and was associated with a significant reduction in myocardial injury and blood loss (page 2504, column 2 in particular). Blood loss in bypass procedures is known to be associated with hemolysis, as evidenced by Claque (Abstract in particular). Based upon the teachings of Fitch in light of the teachings of Reiter, the artisan would recognize that this may be due to the reduced capacity for vasodilation due to the lack of NO caused by free hemoglobin resulting from hemolysis.

It would have been prima facie obvious to a person having ordinary skill in the art at the time the invention was made to treat subjects experience hemolytic disease as a result of coronary bypass surgery for nitric oxide deficiency using the anti-C5 antibody h5G1.1-scFv. One would have been motivated to combine the teachings with a reasonable expectation of success by the teachings of Vakeva that treatment of ischemia with anti-C5 antibody greatly reduced hemolysis, the teachings of Reiter that free hemoglobin resulting from hemolysis during cardiopulmonary bypass procedures greatly reduces NO levels in serum

and the teachings of Fitch that treatment of bypass patients with h5G1.1-scFv antibody reduced cell injury and blood loss.

Claims 112-114 are included because, while the references are silent about the proportion of type III red blood cells, silence about a particular property does not necessarily constitute absence of that property. Also, claims 115-117 are included because, while the references are silent about the platelet counts in a subject, silence about a particular property does not necessarily constitute absence of that property. Furthermore claims 118-120 are included because, while the references are silent about the reticulocyte counts in a subject, silence about a particular property does not necessarily constitute absence of that property. The office does not have the facilities and resources to provide the factual evidence needed in order to establish that there is a difference between the materials, i.e., that the claims are directed to new materials and that such a difference would have been considered unexpected by one of ordinary skill in the art, that is, the claimed subject matter, if new, is unobvious. In the absence of evidence to the contrary, the burden is on the Applicant to prove that the claimed materials are different from those taught by the prior art and to establish patentable differences. See In re Best 562F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray 10 USPQ 2d 1922 (PTO Bd. Pat. App. & Int. 1989).

Conclusion

4. No claim is allowed.

5. Any inquiry concerning this communication or earlier communications from the examiner should be directed to F. Pierre VanderVegt whose telephone number is (571) 272-0852. The examiner can normally be reached on M-Th 6:30-4:00 and Alternate Fridays 6:30-3:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on (571) 272-0841. 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.

F. Pierre VanderVegt, Ph.D.
Patent Examiner
February 20, 2007

DAVID A. SAUNDERS
PRIMARY EXAMINER

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/771,552	02/03/2004	Leonard Bell	ALXN-PO1-114	6183

28120 7590 09/26/2006

FISH & NEAVE IP GROUP
ROPES & GRAY LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624

EXAMINER	
VANDERVEGT, FRANCOIS P	

ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 09/26/2006

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

PTO-90C (Rev. 10/03)

Office Action Summary	Application No.	Applicant(s)	
	10/771,552	BELL ET AL.	
	Examiner	Art Unit	
	F. Pierre VanderVegt	1644	

-- 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 1 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)☐ 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

4)☒ Claim(s) *1-171* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-171* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-171 are currently pending.

Election/Restrictions

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-47, 97-108, and 121-171, drawn to a method of treating a subject with hemolytic disease or hemoglobinuria with an anti-C5 antibody, classified in class 424, subclass 145.1.

II. Claims 48-59, drawn to a method of treating a subject with dysphagia with an anti-C5 antibody, classified in class 424, subclass 145.1.

III. Claims 60-71, drawn to a method of treating a subject with erectile dysfunction with an anti-C5 antibody, classified in class 424, subclass 145.1.

IV. Claims 72-84, drawn to a method of reducing thrombosis in a subject with an anti-C5 antibody, classified in class 424, subclass 145.1.

V. Claims 85-96, drawn to a method of increasing the proportion of type III blood cells in a subject with an anti-C5 antibody, classified in class 424, subclass 145.1.

VI. Claims 109-120, drawn to a method of treating a nitric oxide deficiency in a subject with an anti-C5 antibody, classified in class 424, subclass 145.1.

The inventions are distinct, each from the other because of the following reasons:

2. Inventions I-VI are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different designs, modes of operation, and effects (MPEP § 802.01 and § 806.06). In the instant case, the different inventions use the same reagent for the treatment, but each of the conditions in Groups I-VIhas a different etiology and affects a different part of the body. The anti-C5 antibody is administered therefore to achieve different therapeutic goals in each invention.

3. Because these inventions are independent or distinct for the reasons given above and there would be a serious burden on the examiner if restriction is not required because the inventions have acquired a separate status in the art in view of their different classification. Further, a prior art search requires a literature search. It is an undue burden for the Examiner to search more than one invention and restriction for examination purposes as indicated is proper.

4. Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or invention to be examined even though the requirement be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected invention.

The election of an invention or species may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse.

Should applicant traverse on the ground that the inventions or species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions or species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C.103(a) of the other invention.

5. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to F. Pierre VanderVegt whose telephone number is (571) 272-0852. The examiner can normally be reached on M-Th 6:30-4:00 and Alternate Fridays 6:30-3:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on (571) 272-0841. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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F. Pierre VanderVegt, Ph.D.
Patent Examiner
September 19, 2006

CHRISTINA CHAN
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/050,543	02/03/2005	Leonard Bell	ALXN-P02-114	2128

28120 7590 07/09/2010

ROPES & GRAY LLP
PATENT DOCKETING 39/41
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624

EXAMINER	
GAMBEL, PHILLIP	
ART UNIT	**PAPER NUMBER**
1644	

MAIL DATE	DELIVERY MODE
07/09/2010	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)	
	11/050,543	BELL ET AL.	
	Examiner	Art Unit	
	Phillip Gambel	1644	

-- 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.

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Status

1)☒ Responsive to communication(s) filed on *06/26/2008, 10/02/2008*.

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

3)☐ 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

4)☒ Claim(s) *1-135* is/are pending in the application.

4a) Of the above claim(s) *1-45,48,51-75,78,81-98,101,106 and 109-130* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *46,47,49,50,76,77,79,80,99, 100,102-105,107,108 and 131-135* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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)).

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3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *12/30/2008, 01/20/2010*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. The examiner of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Technology Center 1600, Art Unit 1644.

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office Action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/02/2008 has been entered.

Applicant's amendment, filed 06/26/2008, has been entered
Claims 47, 79, 102 and 107 have been amended.

Claims 1-135 are pending.

Claims 46, 47, 49, 50, 76, 77, 79, 80, 99, 100, 102-105, 107, 108 and 131-135 are under consideration in the instant application as they read on the elected invention.

Claims 1-45, 48, 51-75, 78, 81-98, 101, 106 and 109-130 have been withdrawn from consideration as they read on non-elected inventions/species.

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

4. The application is required to be reviewed and all spelling, TRADEMARKS, and like errors corrected. Appropriate corrections are required.

Trademarks should be capitalized or accompanied by the ® or ™ symbol wherever they appear and be accompanied by the generic terminology. Although the use of trademarks is permissible in patent applications, the proprietary nature of the trademarks should be respected and every effort made to prevent their use in any manner, which might adversely affect their validity as trademarks.

The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP 608.01.
For example, see page 40 of the specification.

Appropriate corrections are required

5. The following is a quotation of the first paragraph of 35 U.S.C. § 112:
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 49 and 99 are rejected under 35 U.S.C. § 112, first paragraph, as the specification does not contain a written description of the claimed invention, in that the disclosure does not reasonably convey to one skilled in the relevant art that the inventor(s) had possession of the claimed invention at the time the application was filed.
The specification as originally filed does not provide support for the invention as now claimed:

The specification as originally filed does not provide support for the invention as now claimed:
(A) "within 38% of the upper limit of normal" as recited in claim 99 and
(B) "PNH type III granulocytes" recited in claim 49.

As noted previously, claim 99 has been amended to recite "38%" in place of the original "20%" limitation. The specification as originally filed discloses only reduction of LDH levels to within 20% of the upper limit of normal at page 23, lines 6-9. Applicant has asserted that the recitation of 38% in the claim is supported by the specification as originally filed in the data of Figure 1A. The Figure in question depicts data from treated patients showing ranges of LDH levels pre- and post-treatment. The Figure "38%" is a post-treatment value obtained from within one of these ranges. The data in the Figure does not specifically lead to a teaching of 38% and there is no teaching in the specification to indicate that this is a preferred value from within the range. Picking a specific value from within a disclosed range creates a new, previously undisclosed, sub-genus and this constitutes new matter.
With respect to the recitation of "38%", applicant's arguments, filed 06/26/2008, submits the following.

> Although the embodiment referred to by the Examiner on page 23, lines 6-9 does not state "38%" this number is specifically taught by the data in Figure 1A. Specifically, Figure 1A shows the upper limit of normal (uln) is 480 U/L, and the treated patients showed an average LDH level of 622.4 + 41.1 at 64 weeks. Thus, the maximum LDH level of treated patients was 38.2% above the uln. Therefore, the upper range of the LDH levels of treated patients is reduced to within 38% of the uln. One of ordinary skill in the art could have derived this number unambiguously from Figure 1A and 38% defines the range rather than being within the range as the Examiner suggests. Accordingly, no new matter has been introduced.

In contrast to applicant's assertions, applicant is relying upon a specific example determined to be 38.2%, while the claims are now drawn to range as well as a specific point that differs from the specific example relied upon by applicant (e.g., "38%" versus "38.2%").

Further, it cannot be said that a subgenus is necessarily described by a genus encompassing it and a species upon which it reads. See In re Smith 173 USPQ 679, 683 (CCPA 1972) and MPEP 2163.05.

Upon a review of the prosecution, it is noted that claim 49 had been amended to change PNH type III granulocyte clones" to PNH type III granulocytes" (see applicant's amendment, filed 01/10/2008).

Applicant relied upon the following paragraph from page 24 of the specification.

Methods of reducing one or more symptoms associated with PNH or other hemolytic diseases are also within the scope of the present disclosure. Such symptoms include, for example, abdominal pain, fatigue, dyspnea and insomnia. Symptoms can be the direct result of lysis of red blood cells (e.g., hemoglobinuria, anemia, fatigue, low red blood cell count, etc.) or the symptoms can result from low nitric oxide (NO) levels in the patient's bloodstream (e.g., abdominal pain, erectile dysfunction, dysphagia, thrombosis, etc.). It has recently been reported that almost all patients with greater than 40% PNH type III granulocyte clone have thrombosis, abdominal pain, erectile dysfunction and dysphagia, indicating a high hemolytic rate (see Moyo et al., British J. Haematol. 126:133-138 (2004)).

Rather than claiming "PNH type III granulocytes" generically, the original claims and the instant specification as-filed limits the cells to "PHN type III granulocyte clones".
By deleting "clones", it appears that applicant has altered the cell populations recited in the claims and described in the specification.

The specification does not provide sufficient blazemarks nor direction for the instant methods encompassing the above-mentioned "limitations" as currently recited. The instant claims now recite limitations which were not clearly disclosed in the specification as-filed, and now change the scope of the instant disclosure as-filed. Such limitations recited in the present claims, which did not appear in the specification, as filed, introduce new concepts and violate the description requirement of the first paragraph of 35 U.S.C. 112.

Applicant is required to cancel the new matter in the response to this Office Action.

Alternatively, applicant is invited to provide sufficient written support for the "limitations" indicated above.
See MPEP 714.02 and 2163.06

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

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

8. 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. §103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. § 103(c) and potential 35 U.S.C. §§ 102(f) or (g) prior art under 35 U.S.C. § 103(a).

9. Claims 46, 47, 49, 50, 76, 77, 79, 80, 99, 100, 102-105, 107, 108 and 131-135 are rejected under 35 U.S.C. § 102(a)(b) as being anticipated by Hillmen et al. (Blood 102: 1858, 2003) (1449; #CD) (see entire document).

Since the public availability of this document is not clear and given that certain claims are subject to a rejection under 35 USC 112, first paragraph, written description / new matter; this rejection is being applied under 35 U.S.C. § 102(a)(b).

Hillmen et al. teach methods of treating paroxysmal nocturnal hemoglobulinuria (PNH), intravascular hemolysis and venous thrombosis, with an anti-C5 antibody and in certain instances with erythropoietin as well, including improvement in biochemical / biological parameters associated with LDH and PNH type III cells (see entire document).

It does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure

Although the reference is silent about certain specific claimed limitations per se of a patient having paroxysmal nocturnal hemoglobulnuria (PNH) (e.g., see claims 49-50, 79-80, 99, 10—104, 107-108, 135),
it does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure. See Bristol-Myers Squibb Company v. Ben Venue Laboratories 58 USPQ2d 1508 (CAFC 2001). "{i}t is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again

patentable". In re Woodruff, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990). The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145.

On this record, it is reasonable to conclude that the same PNH patients are being administered the active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., LDH and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to administer anti-C5 antibodies to treat PNH patients.

10. Claims 46, 47, 49, 50, 76, 77, 79, 80, 99, 100, 102-105, 107, 108 and 131-135 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hillmen et al. (Blood 102: 1858, 2003) (1449; #CD) AND/OR Hillmen (44th ASH Annual Meeting Abstract, Blood, Vol. 100, 2002) (1449; #CE)in view of Meyers et al. (Int. J. Hematol. 77: 125-132, 2003) and as evidenced by Garba et al. (Singapore Med. J. 46(11):632-634, 2005) (892; of record) with respect to normal LDH levels.

Hillmen et al. (2003) teach methods of treating paroxysmal nocturnal hemoglobulinuria (PNH), which is characterized by intravascular hemolysis and venous thrombosis with an anti-C5 antibody and in certain instances with erythropoietin as well, including improvement in biochemical / biological parameters associated with LDH and PNH type III cells (see entire document).

Hillmen (2002) teaches that paroxysmal nocturnal hemoglubinuria (PNH) is characterized by intravascular hemolysis and venous thrombosis. Hillmen teaches that the hemolysis is complement-mediated and due to the deficiency of complement regulatory proteins on red cells, particularly the terminal complement inhibitor CD59 (see entire document). Hillmen teaches treating transfusion-dependent PNH patients with ECULIZUMAB, a humanized anti-C5 antibody (see entire publication). Hillmen teaches that the biological parameters of hemolysis dramatically improved or normalized for all patients within a week of starting therapy. Hillmen further teaches that mean LDH decreased greater than 50%, from 4494 +/- 3380 units/liter to 636 +/- 166 units/liter.

Hillmen (2002) does not teach treating a subject with EPO.

The mean level of LDH in the PNH patients was more than 1.5 times the upper limit of a normal LDH level in a person, as evidenced by Garba, who discloses that normal LDH in a person is 247.10 +/- 19.0 units/liter (Abstract in particular). The prior art teaching clearly anticipates the claimed invention.

Meyers et al. teach management issues in the treatment of paroxysmal nocturnal hemoglobinuria (see entire document), including the hallmarks of hemolysis and anemia (e.g., see pages 125-128) as well as the treatment of this syndrome with complement inhibitors such as anti-C5 antibodies particularly for the hemolysis of PNH (see Section 2.5, Complement Inhibitors) and erythropoietin (see Section 2.6, Management of Nonhemolytic Anemia).

Given the nature of the clinical manifestations of PNH such as hemolysis and anemia, one of ordinary skill in the art at the time the invention was made would have been motivated to select and combine both anti-C5 antibodies and erythropoietin to address and manage the clinical manifestations of PNH over time. One would have been motivated to combine these therapies with a reasonable expectation of success because each treats a different aspect of the hemolytic disease in the patient and could be reasonably expected to improve the prognosis of the patient more rapidly than either therapy alone. From the teachings of the references, it was apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

"The test of obviousness is not express suggestion of the claimed invention in any or all of the references but rather what the references taken collectively would suggest to those of ordinary skill in the art presumed to be familiar with them." See In re Rosselet, 146 USPQ 183, 186 (CCPA 1965).

"There is no requirement (under 35 USC 103(a)) that the prior art contain an express suggestion to combine known elements to achieve the claimed invention. Rather, the suggestion to combine may come from the prior art, as filtered through the knowledge of one skilled in the art." Motorola, Inc. v. Interdigital Tech. Corp., 43 USPQ2d 1481, 1489 (Fed. Cir. 1997).

An obviousness determination is not the result of a rigid formula disassociated from the consideration of the facts of a case. Indeed, the common sense of those skilled in the art demonstrates why some combinations would have been obvious where others would not. See KSR Int'l Co. v. Teleflex Inc., 82 USPQ2d 1385 (U.S. 2007) ("The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.").

11. Given copending applications encompassing similar subject matter and the restriction set forth herein, applicant is invited to clarify which applications should be subject to rejections under the judicially created doctrine of obviousness-type double patenting.

12. No claim is allowed.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (571) 272-0844. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735.

The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Phillip Gambel/

Primary Examiner
Technology Center 1600
Art Unit 1644
July 6, 2010

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/050,543	02/03/2005	Leonard Bell	ALXN-P02-114	2128

28120 7590 04/02/2008

ROPES & GRAY LLP
PATENT DOCKETING 39/41
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624

EXAMINER	
VANDERVEGT, FRANCOIS P	
ART UNIT	PAPER NUMBER
1644	

MAIL DATE	DELIVERY MODE
04/02/2008	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)	
	11/050,543	BELL ET AL.	
	Examiner	Art Unit	
	F. Pierre VanderVegt	1644	

-- 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 *10 January 2008*.

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

3)☐ 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

4)☒ Claim(s) *1-135* is/are pending in the application.

4a) Of the above claim(s) *1-45,48,51-75,78,81-98,101,106 and 109-130* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *46,47,49,50,76,77,79,80,99,100,102-105,107,108 and 131-135* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *20071009, 20080110*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This application is a continuation-in-part of U.S. Application Serial Number 10/771,552.

New claims 131-135 have been added.

Claims 1-135 are currently pending.

Election/Restrictions

1. **Claims 1-45, 51-75, 81-98 and 109-130 stand withdrawn** from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on July 16, 2007.

2. **Claims 48, 78, 101 and 106 stand withdrawn** from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected species, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on July 16, 2007.

3. Accordingly, **claims 46, 47, 49, 50, 76, 77, 79, 80, 99, 100, 102-105, 107, 108 and 131-135 are the subject of examination** in the present Office Action.

4. In view of Applicant's amendment filed January 10, 2008, only the following grounds of rejection are maintained.

Claim Rejections - 35 USC § 112

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

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

5. Claim 99 stands rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a NEW MATTER rejection.

Claim 99 has been amended to recite 38% in place of the original 20% limitation. The specification as originally filed discloses only reduction of LDH levels to within 20% of the upper limit of normal at page 23, lines 6-9. Accordingly, the recitation of 38% constitutes new matter and must be removed.

Applicant's arguments filed January 10, 2008 have been fully considered but they are not persuasive. Applicant asserts that the recitation of 38% in the claim is supported by the specification as originally filed in the data of Figure 1A. The figure in question depicts data from treated patients showing ranges of LDH levels pre- and post-treatment. The figure "38%" is a post-treatment value obtained from within one of these ranges. The data in the figure does not specifically lead to a teaching of 38% and there is no teaching in the specification to indicate that this is a preferred value from within the range. Picking a specific value from within a disclosed range creates a new, previously undisclosed, sub-genus and this constitutes new matter. Accordingly, the ground of rejection is maintained.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> 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.

6. Claims 49, 79, 102 and 107 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 49, 79, 102 and 107 remain ambiguous and unclear in the recitation of "greater than 40% of said subject's granulocytes are paroxysmal nocturnal hemoglubinuria (PNH) type III granulocytes. It is unclear whether this percentage of granulocytes being type III granulocytes is before or after treatment with the complement inhibitor. Clarification is required.

7. The following represents a new ground of rejection that has been necessitated by Applicant's amendment.

Claim Rejections - 35 USC § 103

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.

8. Claims 46, 47, 50, 76, 77, 80, 100, 103-105 and 108 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen (44th ASH Annual Meeting Abstract, Blood, vol. 100 [2002]; CE on form PTO-1449 filed November 24, 2006, of record), as evidenced by Garba et al. (Singapore Med. J. [2005] 46(11):632-634; U on form PTO-892, of record) in view of Stebler et al. (Exp. Hematol. [1990] 18:1204-1208; V on form PTO-892, newly cited).

Hillmen teaches that paroxysmal nocturnal hemoglubinuria (PNH) is characterized by intravascular hemolysis and venous thrombosis. Hillmen teaches that the hemolysis is complement-mediated and due to the deficiency of complement regulatory proteins on red cells, particularly the terminal complement inhibitor CD59. Hillmen teaches treating transfusion-dependent PNH patients with ECULIZUMAB, a humanized anti-C5 antibody (see entire publication). Hillmen teaches that the biological parameters of hemolysis dramatically improved or normalized for all patients within a week of starting therapy. Hillmen further teaches that mean LDH decreased greater than 50%, from 4494 +/- 3380 units/liter to 636 +/- 166 units/liter. The mean level of LDH in the PNH patients was more than 1.5 times the upper limit of a normal LDH level in a person, as evidenced by Garba, who discloses that normal LDH in a person is 247.10 +/- 19.0 units/liter (Abstract in particular). The prior art teaching clearly anticipates the claimed invention.

Hillmen does not teach treating a subject with EPO.

It is noted that the specification teaches the treatment with EPO as a combination therapy with the complement inhibitor. The specification discloses that the purpose of the EPO is to stimulate hematopoiesis and does not disclose that EPO modifies the activity of the complement inhibitor in any way. Accordingly, there is no disclosure that there any unobvious effects of the EPO on the inhibitor or vice versa.

Stebler teaches the use of high doses of recombinant human EPO to treat the anemia in patients with PNH (see entire document).

It would have been prima facie obvious to a person having ordinary skill in the art at the time the invention was made to treat a patient with PNH with ECULIZUMAB to inhibit hemolysis as taught by Hillmen and to simultaneously treat that patient with EPO to stimulate hematopoiesis and alleviate anemia as taught by Stebler. One would have been motivated to combine these therapies with a reasonable expectation of success because each treats a different aspect of the hemolytic disease in the

patient and could be reasonably expected to improve the prognosis of the patient more rapidly than either therapy alone.

Conclusion

9. No claim is allowed.

10. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is 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 date of this final action.

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to F. Pierre VanderVegt whose telephone number is (571)272-0852. The examiner can normally be reached on M-Th 6:30-4:00 and Alternate Fridays 6:30-3:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Eileen O'Hara can be reached on (571) 272-0878. 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.

F. Pierre VanderVegt, Ph.D. /PV/
Patent Examiner
March 27, 2008

/David A Saunders/
Primary Examiner, Art Unit 1644

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/050,543	02/03/2005	Leonard Bell	ALXN-P02-114	2128

28120 7590 10/10/2007
ROPES & GRAY LLP
PATENT DOCKETING 39/41
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624

EXAMINER	
VANDERVEGT, FRANCOIS P	

ART UNIT	PAPER NUMBER
1644	

MAIL DATE	DELIVERY MODE
10/10/2007	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)	
	11/050,543	BELL ET AL.	
	Examiner	Art Unit	
	F. Pierre VanderVegt	1644	

-- 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 *16 July 2007*.

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

3)☐ 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

4)☒ Claim(s) *1-130* is/are pending in the application.

4a) Of the above claim(s) *1-45,48,51-75,78,81-98,101,106 and 109-130* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *46,47,49,50,76,77,79,80,99,100,102-105,107 and 108* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *20050511, 20060824, 20061124*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This application is a continuation-in-part of U.S. Application Serial Number 10/771,552.

Claims 1-130 are currently pending.

Election/Restrictions

1. Applicant's election with traverse of Group V, claims 46-50, in the reply filed on July 16, 2007 is acknowledged. The traversal is on the ground(s) that claims 76-80 and 99-108 should have also been included in this group because they are drawn to the same method. Upon further review of the claims in light of Applicant's remarks, the Examiner concurs and will rejoin the aforementioned claims with the invention of Group V.

Claims 1-45, 51-75, 81-98 and 109-130 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on July 16, 2007.

2. Applicant's election of the species "antibody" in the reply filed on July 16, 2007 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claims 48, 78, 101 and 106 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected species, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on July 16, 2007.

3. Accordingly, **claims 46, 47, 49, 50, 76, 77, 79, 80, 99, 100, 102-105, 107 and 108 are the subject of examination** in the present Office Action.

Claim Rejections - 35 USC § 112

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

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

4. Claim 99 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the

specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a NEW MATTER rejection.

Claim 99 has been amended to recite 38% in place of the original 20% limitation. The specification as originally filed discloses only reduction of LDH levels to within 20% of the upper limit of normal at page 23, lines 6-9. accordingly, the recitation of 38% constitutes new matter and must be removed.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> 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.

5. Claims 49, 79, 102 and 107 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 49, 79, 102 and 107 are ambiguous and unclear in the recitation of the abbreviation "PNH." The first recitation of an abbreviation in a line of claims should be in addition to a recitation of the full term, as the same abbreviation can be used to identify other cells or conditions. This ground of rejection can be overcome by amending the claim to recite --paroxysmal nocturnal hemoglubinuria (PNH)-- in place of the recitation of "PNH."

Claims 49, 79, 102 and 107 are ambiguous and unclear in the recitation of "subject has a greater than 40% PNH type III granulocytes clone. It is not clear what is exactly is supposed to be greater than 40% in the subject. Type III cells? Granulocytes? The ratio of granulocytes to type III cells? What is a "40% PNH type III granulocyte clone?" Clarification is required.

Claim Rejections - 35 USC § 102

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

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

6. Claims 46, 47, 50, 76, 77, 80, 100, 103-105 and 108 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Hillmen (44th ASH Annual Meeting Abstract, Blood, vol. 100 [2002]; CE on form PTO-1449 filed November 24, 2006), as evidenced by Garba et al. (Singapore Med. J. [2005] 46(11):632-634; U on form PTO-892).

Hillmen teaches that paroxysmal nocturnal hemoglubinuria (PNH) is characterized by intravascular hemolysis and venous thrombosis. Hillmen teaches that the hemolysis is complement-mediated and due to the deficiency of complement regulatory proteins on red cells, particularly the terminal complement inhibitor CD59. Hillmen teaches treating transfusion-dependent PNH patients with ECULIZUMAB, a humanized anti-C5 antibody 9see entire publication). Hillmen teaches that the biological parameters of hemolysis dramatically improved or normalized for all patients within a week of starting therapy. Hillmen further teaches that mean LDH decreased greater than 50%, from 4494 +/- 3380 units/liter to 636 +/- 166 units/liter. The mean level of LDH in the PNH patients was more than 1.5 times the upper limit of a normal LDH level in a person, as evidenced by Garba, who discloses that normal LDH in a person is 247.10 +/- 19.0 units/liter (Abstract in particular). The prior art teaching clearly anticipates the claimed invention.

Conclusion

7. No claim is allowed.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to F. Pierre VanderVegt whose telephone number is (571) 272-0852. The examiner can normally be reached on M-Th 6:30-4:00 and Alternate Fridays 6:30-3:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on (571) 272-0841. 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.

F. Pierre VanderVegt, Ph.D. /PV/
Patent Examiner
September 26, 2007

David A. Saunders
DAVID A. SAUNDERS
PRIMARY EXAMINER

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/050,543	02/03/2005	Leonard Bell	ALXN-P02-114	2128

28120 7590 06/11/2007

FISH & NEAVE IP GROUP
ROPES & GRAY LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624

EXAMINER	
VANDERVEGT, FRANCOIS P	

ART UNIT	PAPER NUMBER
1644	

MAIL DATE	DELIVERY MODE
06/11/2007	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)	
	11/050,543	BELL ET AL.	
	Examiner	Art Unit	
	F. Pierre VanderVegt	1644	

-- 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 1 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)☐ 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

4)☒ Claim(s) *1-130* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-130* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This application is a continuation-in-part of U.S. Application Serial Number 10/771,552.

Claims 1-130 are currently pending.

Election/Restrictions

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-19, 86-92 and 109-130, drawn to a method for treating a hemolytic disease in a subject comprising administering a complement-affecting compound to the subject, wherein the subject's need for transfusion is reduced or eliminated, classified in class 424, subclass 278.1.

II. Claims 20-35, drawn to a method for reducing abdominal pain in a subject comprising administering a complement-affecting compound to the subject, classified in class 424, subclass 278.1.

III. Claims 36-40, 51-55, 76-80, and 99-108, drawn to a method for treating a hemolytic disease comprising administering a complement-affecting compound to the subject wherein there is a reduction in hemoglobinuria, classified in class 424, subclass 278.1.

IV. Claims 41-45 and 81-85, drawn to a method for restoring nitric oxide homeostasis or reducing nitrous oxide deficiency in a subject comprising administering a complement-affecting compound to the subject, classified in class 424, subclass 278.1.

V. Claim46-50, drawn to a method for reducing hemolysis disease by reducing LDH levels in the bloodstream comprising administering a complement affecting compound to the subject, classified in class 424, subclass 278.1.

VI. Claims 56-60, drawn to a method for reducing dysphagia in a subject comprising administering a complement-affecting compound to the subject, classified in class 424, subclass 278.1.

VII. Claims 61-65, drawn to a method for reducing erectile dysfunction in a subject comprising administering a complement-affecting compound to the subject, classified in class 424, subclass 278.1.

VIII. Claims 66-70, drawn to a method for reducing thrombosis in a subject comprising administering a complement-affecting compound to the subject, classified in class 424, subclass 278.1.

IX. Claims 71-75, drawn to a method for increasing the proportion of Type III red blood cells in a subject's blood comprising administering a complement affecting compound to the subject, classified in class 424, subclass 278.1.

X. Claims 93-98, drawn to a method for treating a hemolytic disease in a subject comprising administering a complement affecting compound and a compound that increases hematopoiesis to the subject, classified in class 424, subclass 278.1.

2. The inventions are distinct, each from the other because of the following reasons:

Inventions I-X are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different designs, modes of operation, and effects (MPEP § 802.01 and § 806.06). In the instant case, the different inventions use the same reagent for the treatment, but each of the conditions in Groups I-X has a different etiology and/or affects a different part of the body. The anti-C5 antibody is administered therefore to achieve different therapeutic goals in each invention.

3. These inventions are independent or distinct for the reasons given above and there would be a serious burden on the examiner if restriction is not required because the inventions have acquired a separate status in the art due to their recognized divergent subject matter. Further, a prior art search requires a literature search. It is an undue burden for the Examiner to search more than one invention and restriction for examination purposes as indicated is proper.

4. This application contains claims directed to the following patentably distinct species:

a. antibodies,
b. soluble complement inhibitory compounds,
c. proteins, protein fragments, peptides,
d. small molecules,
e. RNA aptamers, L-RNA aptamers,
f. spiegelmers,
g. antisense compounds,
h. serine protease inhibitors,
i. double stranded RNA,
j. small interfering RNA,
k. locked nucleic acid inhibitors,
l. and peptide nucleic acid inhibitors.

The species are independent or distinct because each of the species has a different structure and different mode of action.

Irrespective of which group is chosen, Applicant is further required under 35 U.S.C. 121 to elect a single disclosed species for prosecution on the merits to which the claims shall be restricted if no

generic claim is finally held to be allowable. Currently, claims 1, 2, 4, 6, 7, 9-20, 25-37, 39-42, 44-47, 49-52, 54-57, 59-62, 64-67, 69-72, 74-77, 79-82, 84-87, 89-94, 96-100, 102-105, 107-112, 115, and 117-130 are generic.

Applicant is advised that a reply to this requirement must include an identification of the species that is elected consonant with this requirement, and a listing of all claims readable thereon, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141. If claims are added after the election, applicant must indicate which are readable upon the elected species. MPEP § 809.02(a).

5. Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or invention to be examined even though the requirement be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected invention.

The election of an invention or species may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse.

Should applicant traverse on the ground that the inventions or species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions or species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C.103(a) of the other invention.

6. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to F. Pierre VanderVegt whose telephone number is (571) 272-0852. The examiner can normally be reached on M-Th 6:30-4:00 and Alternate Fridays 6:30-3:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on (571) 272-0841. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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F. Pierre VanderVegt, Ph.D.
Patent Examiner
June 4, 2007

David A Saunders

DAVID A. SAUNDERS
PRIMARY EXAMINER

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.
11/595,118	11/08/2006	Leonard Bell	ALXN-P03-114	6763

28120 7590 01/21/2011

ROPES & GRAY LLP
IPRM - Floor 43
PRUDENTIAL TOWER
800 BOYLSTON STREET
BOSTON, MA 02199-3600

EXAMINER	
GAMBEL, PHILLIP	
ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
01/21/2011	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):

USPatentMail@ropesgray.com
USPatentMail2@ropesgray.com

Office Action Summary	Application No.	Applicant(s)	
	11/595,118	BELL ET AL.	
	Examiner	Art Unit	
	Phillip Gambel	1644	

-- 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 *03 November 2010*.

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

3)☐ 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

4)☒ Claim(s) *1-191* is/are pending in the application.

4a) Of the above claim(s) *1-163 and 168-191* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *164-167* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *10/09/2007, 01/25/2008, 01/05/2009, 01/20/2010, 11/03/2010*
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election of Group II (claims 164-167) for continued examination in the Response to Restriction Requirement, filed 11/03/2010 is acknowledged.

Applicant's indication that an election of species as it reads on Group II is not necessary (at this time) is acknowledged.

Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (see MPEP 818.03(a)).

Therefore, claims 164-167 being acted upon as the elected invention.

Claims 1-163 and 168-191 have been withdrawn as being drawn to the non-elected Group and its corresponding species

2. The effective filing date of the instant claims is deemed to be the filing date of the instant application USSN 11/595,118, filed 11/08/2206, as the previous priority applications do not provide sufficient written support for claims 164-167 as follows.

A method of determining whether a subject having a hemolytic disorder is susceptible to thrombosis comprising measuring the PNH granulocyte clone size of said subject, wherein if the clone size is greater than O. 1% then said subject is susceptible to thrombosis.

If applicant disagrees, applicant should present a detailed analysis as to why the claimed subject matter has clear support in the earlier priority applications.

Applicant is reminded that such priority for the instant limitations requires written description and enablement under 35 U.S.C. § 112, first paragraph.

3. If applicant desires priority under 35 U.S.C. 119(e) / 120 based upon a previously filed application, specific reference to the earlier filed application must be made in the instant application. For benefit claims under 35 U.S.C. 120, 121 or 365(c), the reference must include the relationship (i.e., continuation, divisional, or continuation-in-part) of the applications. This should appear as the first sentence of the specification following the title, preferably as a separate paragraph unless it appears in an application data sheet. The status of nonprovisional parent application(s) (whether patented or abandoned) should also be included. If a parent application has become a patent, the expression "now Patent No. _____" should follow the filing date of the parent application. If a parent application has become abandoned, the expression "now abandoned" should follow the filing date of the parent application.

Applicant should update the status of the priority documents on page 1 of the instant specification.

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

5. The application is required to be reviewed and all spelling, TRADEMARKS, and like errors corrected. Appropriate corrections are required.

Trademarks should be capitalized or accompanied by the ® or ™ symbol wherever they appear and be accompanied by the generic terminology. Although the use of trademarks is permissible in patent applications, the proprietary nature of the trademarks should be respected and every effort made to prevent their use in any manner, which might adversely affect their validity as trademarks.

Appropriate corrections are required

6. For examination purposes, it is noted the measuring the PNH granulocyte clone size refers to measuring the number or PNH granulocytes and not measuring the physical size of PNH granulocytes.

7. Claims 164-167 are objected to as "PNH" should be recited as "paroxysmal nocturnal hemoglobinuria" upon first time usage for clarity.

8. Claim 164-167 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 164-167 are indefinite in the recitation of "wherein the clone is greater than 0.1% / 1% / 10% / 50%" because a referenced control is not indicated.

Applicant is invited to consider amending the claims to include "of the total granulocyte count" as described on page 12, paragraph 4 of the instant specification to obviate this rejection.

Applicant is reminded that the amendment must point to a basis in the specification so as not to add any new matter. See MPEP 714.02 and 2163.06

9. The following is a quotation of the first paragraph of 35 U.S.C. § 112:
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

10. Claims 164-167 are rejected under 35 U.S.C. § 112, first paragraph, because the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention.

The instant encompass "determining whether a subject is susceptible to thrombosis" based upon measuring the related clone size of PNH granulocytes" as follows.

164. A method of determining whether a subject having a hemolytic disorder is susceptible to thrombosis comprising measuring the PNH granulocyte clone size of said subject, wherein if the clone size is greater than 0.1% then said subject is susceptible to thrombosis.

165. The method of claim 164, wherein said clone size is greater than 1%.

166. The method of claim 164, wherein said clone size is greater than 10%.

167. The method of claim 164, wherein said clone size is greater than 50%.

Moyo et al. (British Journal of Haematology 126: 133-138, 2004) (1449; #CY) teach studied and evaluated patients with paroxysmal nocturnal hemoglobinuria (PNH) using sensitive and specific diagnostic assays and concluded that their data not only confirmed that the size of PNH clones, including PNH clones as measured in granulocytes, correlates with the risk of thrombosis, but also suggests a correlation of PNH clones to more symptomatic PNH (see entire document, including Abstract, Materials and Methods, Results, including Figures 1-4, and Discussion).

Moyo et al. found a strong association between PNH clones as measured in granulocytes and thrombosis, where the cutoff between the percentage of PNH granulocytes and thrombosis was 61% and the odds ratio for thrombosis was 1:64 for a 10% change in the size of the PNH granulocyte clones (e.g., see Results, particularly, page 135, column 2, paragraphs 1-2).

Such observations would indicate that there is a lack of correlation of measuring the clone size of PNH granulocytes and thrombosis, when the clone size is less than 61% relative to total granulocyte size.

Therefore, there is a lack of predictability of correlating PNH granulocyte size and susceptibility of thrombosis when the clone is 0.1% , 1% , 10% or 50%" as recited in the instant claims.

In turn,, the specification does not enable one of skill in the art, at the time the invention was made, to practice the claimed methods of a lack of correlation of measuring the clone size of PNH granulocytes and thrombosis, when the clone size is less than 61% relative to total granulocyte size.

Also, a medical diagnosis is a process of identifying a medical condition or disease by its signs, symptoms, and from the results of various diagnostic procedures.

Several variables are used in evaluating the predictability of detection or diagnostic assays.

These include diagnostic specificity and sensitivity and positive and negative predictive values.

The sensitivity of an assay reflects the fraction of those subjects with a specific disease that the assay correctly identifies as positive; while the specificity of an assay reflects the fraction of those subjects without the disease that the assay correctly identifies as negative.

The positive predictive value refers to the probability that an individual with a positive test result has the diseases; while the negative predictive value refers to the probability that an individual with a negative test result does not have the disease.

There is an inverse relationship between the sensitivity and specificity, which is related to the assigned cutoff value that is used for a particular test to segregate diseased populations from those with no disease.

In the absence of objective evidence to the contrary and keeping with the lack of predictability of a lack of correlating measuring the clone size of PNH granulocytes and thrombosis, when the clone size is less than 61% relative to total granulocyte size as well as the differences and multiple variables associated with different hemolytic disorders wherein there is a susceptibility to thrombosis encompassed by the claimed methods,

there is insufficient disclosure on how to make and use the claimed methods to a lack of correlate measuring the clone size of PNH granulocytes and thrombosis, when the clone size is less than 61% relative to total granulocyte size.

Given the unpredictability of the art in a lack of correlation of measuring the clone size of PNH granulocytes and thrombosis, when the clone size is less than 61% relative to total granulocyte size,

the experimentation left to those skilled in the art, would be unnecessarily, and improperly, extensive and undue.

In view of the lack of predictability of the art to which the invention pertains the lack of established protocols for correlating susceptibility to thrombosis based upon the presence alone of PNH granulocytes, undue experimentation would be required to practice the claimed methods of diagnosing / determining whether a subject is susceptible to thrombosis by determining PNH granulocytes of 0.1%-50% relative to controls with a reasonable expectation of success, absent a specific and detailed description in applicant's specification of how to effectively practice the claimed methods and absent working examples providing evidence which is reasonably predictive that the claimed methods are effective for determining susceptibility to thrombosis based upon a broad range of PNH granulocyte sizes relative to control encompassed by the claimed methods.

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

A person shall be entitled to a patent unless --

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

12. Claims 164-167 are rejected under 35 U.S.C. § 102(b) as anticipated by Moyo et al. (British Journal of Haematology 126: 133-138, 2004) (1449; #CY) (see entire document).

Moyo et al. teach studied and evaluated patients with paroxysmal nocturnal haemoglobinuria (PNH) using sensitive and specific diagnostic assays and concluded that their data not only confirmed that the size of PNH clones, including PNH clones as measured in granulocytes, correlates with the risk of thrombosis, but also suggests a correlation of PNH clones to more symptomatic PNH (see entire document, including Abstract, Materials and Methods, Results, including Figures 1-4, and Discussion).

Note, too, that Moyo et al. discuss validating the work of others, who showed that large PNH clones are predictive of thrombosis (see Discussion).

While Moyo et al. does not describe "wherein the clone is greater than 0.1% / 1% / 10% / 50%" explicitly,

Moyo et al. found a strong association between PNH clones as measured in granulocytes and thrombosis, where the cutoff between the percentage of PNH granulocytes and thrombosis was 61% and the odds ratio for thrombosis was 1:64 for a 10% change in the size of the PNH granulocyte clones (e.g., see Results, particularly, page 135, column 2, paragraphs 1-2).

These values anticipate the broad ranges encompasss the claimed limitation(s) "wherein the clone is greater than 0.1% / 1% / 10% / 50%".

Regarding the recitation of "identifying a subject having or being at risk of having a condition related to an excessive T cell mediated immune response",

it is noted that this "observing" step or clause does not recite any additional active method steps, but simply states a characterization or conclusion of the results of those steps or may be performed entirely in the human mind is obviously not tied to any machine and does not transform any article into a different state or thing.

Therefore, the "identifying a subject" step or clause simply recites or choosing the intended patient population for the claimed methods taught by the prior art, given the broadest reasonable interpretation of the claims.

13. No claim allowed.

14. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (571) 272-0844. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735.

The fax number for the organization where this application or proceeding is assigned is 571-272-8300.

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/Phillip Gambel/
Primary Examiner
Technology Center 1600
Art Unit 1644
January 18, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/595,118	11/08/2006	Leonard Bell	ALXN-P03-114	6763

28120 7590 10/05/2010

ROPES & GRAY LLP
PATENT DOCKETING 39/41
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624

EXAMINER	
GAMBEL, PHILLIP	

ART UNIT	PAPER NUMBER
1644	

MAIL DATE	DELIVERY MODE
10/05/2010	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)	
	11/595,118	BELL ET AL.	
	Examiner	Art Unit	
	Phillip Gambel	1644	

-- 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 *1* 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 *20 September 2010*.

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

3)☐ 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

4)☒ Claim(s) *1-191* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-191* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *09/20/2010* .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-191 are pending.

2. On 08/05/2010 and with reminders on 08/30/2010 and 09/20/2010,
Applicant's representative Xuqiong Wu requested that a new Restriction be set forth to clarify the discrepancies between the Restriction mailed 04/03/2009 and the Restriction mailed 07/07/2010 in order to clarify the Groups and the Election of Species.

The examiner agreed to issue a new Restriction to clarify the Groups and the Election of Species.

See attached Interview Summary.

3. The previous Restriction Requirements, mailed 04/03/2009 and 07/07/2010, have been withdrawn

From a review of the specification, it appears that all of the claims are drawn to methods of treating hemolytic diseases, which encompass various symptoms such as hemoglobinuria, anemia, dysphagia, fatigue, erectile dysfunction, recurrent abdominal pain and thrombosis (e.g., see pages 15-16 of the Detailed Description).
Therefore, setting forth the claimed invention in terms of Groups as done previously appears inconsistent with treating hemolytic diseases.

Claims 1-191 are pending and currently under Election of Species set forth herein.

The examiner apologizes for any inconvenience to applicant in this matter/

4. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claim 1-163 and 168-191, drawn to a method of reducing the occurrence of thrombosis in a subject, classified in class 424/130.1 and 514, subclass 2, for example.

From a review of the specification, it appears that all of the claims of this Group are drawn to methods of treating hemolytic diseases, which encompass various symptoms such as hemoglobinuria, anemia, dysphagia, fatigue, erectile dysfunction, recurrent abdominal pain and thrombosis (e.g., see pages 15-16 of the Detailed Description).

In turn, claims 1-163 and 168-191 are subject to an Election of Species set forth herein.

II. Claims 164-167, drawn to a method of determining whether a subject having a hemolytic disorder is susceptible to thrombosis by measuring PNH granulocyte size, classified in Class 435, subclass 7.1.

5. The inventions are distinct, each from the other because of the following reasons:

Inventions I and II are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different designs, modes of operation, and effects (MPEP § 802.01 and § 806.06).

In the instant case, the different inventions are drawn to methods of treatment in Group I and II and the method of diagnosing in Group II rely upon different ingredients, methods steps and endpoints.

The therapeutic / diagnostic methods do not require the practice of one another.

Also, each Group would require a separate search of the literature.

7. Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and examination burden if restriction were not required because one or more of the following reasons apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;
(b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;
(c) the inventions require a different field of search (for example, searching different classes/subclasses or electronic resources, or employing different search queries);
(d) the prior art applicable to one invention would not likely be applicable to another invention;
(e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected invention.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected invention.

If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

8. Election of Species.

This application is directed to the following species.
A) administering a compound as set forth in claims 2 and 7 for example alone or
B) administering a compound as set forth in claims 2 and 7 for *example in combination with a second compound* as set forth in claim 31 for example.

The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

The species are distinct because they require different methods steps, ingredients and endpoints.

Further, these species of first and second compounds are distinct because their structures, physicochemical properties and modes of action differ action to such an extent and require non-coextensive searches to such an extent that they are considered patentable distinct. Further, these molecules do not share a substantial structural feature essential to a common utility do not have common structure to a common utility. Therefore, they are separate and patentably distinct species.

Applicant is required under 35 U.S.C. § 121 to elect a single disclosed species of (A) or (B) AND elect a specific ultimate species of
a first compound as set forth in (A) *(e.g., anti-C5 antibody or a specific RNA aptamer)* and
a specific ultimate species a second *compound of (B) (e.g., EPO, anti-lymphocyte globulin)* if elected herein
for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. For example, claim 1 is generic.

There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

9. In addition to the election of species from Section 8;
then applicant is required to elect a species from the following.

This application contains claims directed to the following patentably distinct species of the claimed invention by electing a specific hemolytic disease and/or a specific symptom of a hemolytic disease.

From a review of the specification, the only hemolytic disease described appears to be paroxysmal nocturnal hemoglobinuria (PNH).

If other hemolytic diseases are described, then applicant should elected a specific hemolytic disease.

In addition, particularly if the only hemolytic disease described is PNH,
then applicant should elect a specific symptom of a hemolytic disease such as hemoglobinuria, anemia, dysphagia, fatigue, erectile dysfunction, recurrent abdominal pain and thrombosis either claimed or disclosed in the instant specification (e.g., see pages 15-16 of the Detailed Descrption).

Alternatively, if applicant deems that all of the symptoms are inherent or obvious in treating patients with treating hemolytic diseases,
Then this aspect of the election of species may be withdrawn.

At this point, it is not clear whether applicant is treating different diseases or patient populations. Applicant is invited to clarify this issue in the interest of compact prosecution.

The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

These species are distinct because these targeted hemolytic diseases and/or symptoms differ in etiologies and therapeutic endpoints. Therefore, they are separate and patentably distinct species.

Applicant is required under 35 U.S.C. § 121 to elect a single disclosed species *(e.g., PNH if electing a hemolytic disease AND anemia, erectile dysfunction for a symptom)* for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable.

There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

10. Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected species, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election of the species may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species.

Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

11. Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed.

12. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. 1.48(b) and by the fee required under 37 C.F.R. 1.17(h).

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (571) 272-0844. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735.

The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Phillip Gambel/
Primary Examiner
Technology Center 1600
Art Unit 1644
September 29, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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GAMBEL, PHILLIP	

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Status

1)☒ Responsive to communication(s) filed on *27 April 2009*.

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

3)☐ 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

4)☒ Claim(s) *1-191* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-191* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. . The examiner of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Technology Center 1600, Art Unit 1644

2. Applicant's Response to Restriction Requirement, filed 04/27/2009, is acknowledged.

However, upon a review of the instant specification, the previous Restriction Requirement, mailed 04/03/2009, has been withdrawn

From a review of the specification, it appears that all of the claims are drawn to methods of treating hemolytic diseases, which encompass various symptoms such as hemoglobinuria, anemia, dysphagia, fatigue, erectile dysfunction, recurrent abdominal pain and thrombosis (e.g., see pages 15-16 of the Detailed Description).

Therefore, setting forth the claimed invention in terms of Groups as done previously appears inconsistent with treating hemolytic diseases.

Claims 1-191 are pending and currently under Election of Species set forth herein.

The examiner apologizes for any inconvenience to applicant in this matter/

3. This application is directed to the following species.
   A) administering a compound as set forth in claims 2 and 7 for example alone or
   B) administering a compound as set forth in claims 2 and 7 for example *in combination with a second compound as set forth in claim 31 for example.*

The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

The species are distinct because they require different methods steps, ingredients and endpoints.

Further, these species of first and second compounds are distinct because their structures, physicochemical properties and modes of action differ action to such an extent and require non-coextensive searches to such an extent that they are considered patentable distinct. Further, these molecules do not share a substantial structural feature essential to a common utility do not have common structure to a common utility. Therefore, they are separate and patentably distinct species.

Applicant is required under 35 U.S.C. § 121 to elect a single disclosed species of (A) or (B) AND elect a specific ultimate species of a first compound as set forth in (A) *(e.g., anti-C5 antibody or a specific RNA aptamer)* and a specific ultimate species a second compound of (B) *(e.g., EPO, antilymphocyte globin)* if elected herein for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. For example, claim 1 is generic.

There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

4. In addition to the election of species from Section 3;
then applicant is required to elect a species from the following.

This application contains claims directed to the following patentably distinct species of the claimed invention by electing a specific hemolytic disease and/or a specific symptom of a hemolytic disease.

From a review of the specification, the only hemolytic disease described appears to be paroxysmal nocturnal hemoglobinuria (PNH).
If other hemolytic diseases are described, then applicant should elected a specific hemolytic disease.

In addition, particularly if the only hemolytic disease described is PNH,
then applicant should elect a specific symptom of a hemolytic disease such as hemoglobinuria, anemia, dysphagia, fatigue, erectile dysfunction, recurrent abdominal pain and thrombosis either claimed or disclosed in the instant specification (e.g., see pages 15-16 of the Detailed Descrption).

Alternatively, if applicant deems that all of the symptoms are inherent or obvious in treating patients with treating hemolytic diseases,
Then this aspect of the election of species may be withdrawn.

At this point, it is not clear whether applicant is treating different diseases or patient populations. Applicant is invited to clarify this issue in the interest of compact prosecution.

The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

These species are distinct because these targeted hemolytic diseases and/or symptoms differ in etiologies and therapeutic endpoints. Therefore, they are separate and patentably distinct species.

Applicant is required under 35 U.S.C. § 121 to elect a single disclosed *species (e.g., PNH if electing a hemolytic disease AND anemia, erectile dysfunction for a symptom)* for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable.

There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

5. Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected species, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election of the species may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species.

Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

6. Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed.

7. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. 1.48(b) and by the fee required under 37 C.F.R. 1.17(h).

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (571) 272-0844. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735.

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/Phillip Gambel/
Primary Examiner
Technology Center 1600
Art Unit 1644
July 6, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

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Status

1)☐ Responsive to communication(s) filed on _____.

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

3)☐ 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

4)☒ Claim(s) *1-191* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-191* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-163, drawn to a method of reducing the occurrence of thrombosis in a subject, classified in class 514, subclass 2, for example.

II. Claims 164-167, drawn to a method of determining whether a subject having a hemolytic disorder is susceptible to thrombosis by measuring PNH granulocyte size, classified in class 435, subclass 7.1.

III. Claims 168-179, drawn to a method of increasing PNH red blood cell mass in a subject comprising inhibiting complement, classified in class 424, subclass 130.1, for example.

IV. Claims 180-191, drawn to a method for treating hemolytic anemia in a subject comprising inhibiting complement, classified in class 424, subclass 130.1 for example.

The inventions are distinct, each from the other because of the following reasons:

2. Inventions I, III, IV and II are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different designs, modes of operation, and effects (MPEP § 802.01 and § 806.06). In the instant case, the different inventions are drawn to methods of treatment in Groups I, III and IV, while Group II is drawn to a method of diagnosing a hemolytic disorder by measuring the size of the PNH granulocyte population in a subject. The diagnostic method does not require the practice of the treatment method and vice-versa.

3. Inventions I and III and IV are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different designs, modes of operation, and effects (MPEP § 802.01 and § 806.06). In the instant case, the different inventions are drawn to methods for the treatment of different conditions, each of which would require a separate search of the literature.

4. Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and examination burden if restriction were not required because one or more of the following reasons apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;

(b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;

(c) the inventions require a different field of search (for example, searching different classes/subclasses or electronic resources, or employing different search queries);

(d) the prior art applicable to one invention would not likely be applicable to another invention;

(e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention**.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected invention.

If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

5. **This application contains claims directed to the following patentably distinct species**:

IN GROUP I, there are three species elections required:

Applicant must elect a subject subset for search selected from the group consisting of patients with a higher than normal LDH level (claims 41-122) and patients with lower than normal nitric oxide level (123-163).

Applicant must further elect a complement inhibitor from the compounds enumerated in claims 7 OR 8.

Applicant must further elect a second compound for treatment of a subject selected from a compound that increases hematopoiesis and an anti-thrombotic compound.

6. The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

Should Applicant elect Group I, Applicant is further required under 35 U.S.C. 121 to elect a single disclosed species for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 11-6, 9-14 and 19-29 are generic.

There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species**, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election of the species may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species.

Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

7. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to F. Pierre VanderVegt whose telephone number is (571)272-0852. The examiner can normally be reached on M-F 9:00-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Eileen O'Hara can be reached on (571) 272-0878. 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.

F. Pierre VanderVegt, Ph.D. /PV/
Patent Examiner
March 27, 2009

/Eileen B. O'Hara/
Supervisory Patent Examiner
Art Unit 1644

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/225,040	05/13/2009	Leonard Bell	ALXN-P04-114	5538

28120 7590 09/22/2011

ROPES & GRAY LLP
IPRM - Floor 43
PRUDENTIAL TOWER
800 BOYLSTON STREET
BOSTON, MA 02199-3600

EXAMINER	
ROGERS, JAMES L	
ART UNIT	**PAPER NUMBER**
1644	

NOTIFICATION DATE	DELIVERY MODE
09/22/2011	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):

USPatentMail@ropesgray.com
USPatentMail2@ropesgray.com

Office Action Summary	Application No.	Applicant(s)	
	12/225,040	BELL ET AL.	
	Examiner	Art Unit	
	JAMES ROGERS	1644	

-- 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 *21 July 2011*.

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

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

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

Disposition of Claims

5)☒ Claim(s) *93-108* is/are pending in the application.

5a) Of the above claim(s) *103-108* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *93-102* 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 *5/13/2009, 1/20/2010, 9/29/2010, 5/10/2010*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Claims 93-108 are pending.

2. Applicant's election with traverse of Group I, claims 93-102, drawn to a pharmaceutical composition comprising a single unit dosage form of an anti-C5 antibody or a C5-binding fragment thereof, wherein the single unit dosage form comprises 300 mg of the anti-C5 antibody or C5-binding fragment thereof, and wherein the anti-C5 antibody or C5-binding fragment thereof inhibits cleavage of C5 into fragments C5a and C5b, filed on 07/21/11, is acknowledged.

Applicant's traversal is on the grounds that the cited reference of Hillmen et al. (N Engl J Med 2004; 350: 552-9) cited in the restriction requirement mailed on 6/1/2011 does not disclose or even suggest the instantly claimed pharmaceutical compositions or the use of the claimed compositions for treating patients afflicted with a hemolytic disease and thus Groups I and II are linked by a unifying inventive technical feature.

This argument was not found persuasive for the same reasons in the restriction requirement mailed 6/1/2011 and for the reasons in the obviousness rejection set forth below.

The requirement is still deemed proper and is therefore made FINAL.

3. Claims 103-108 (non-elected Groups II) are withdrawn from further consideration by the Examiner, 37 C.F.R. § 1.142(b) as being drawn to nonelected inventions/species.

4. Claims 93-102 are under consideration in the instant examination as they read on a method of pharmaceutical composition comprising a single unit dosage form of an anti-C5 antibody or a C5-binding fragment thereof, wherein the single unit dosage form comprises 300 mg of the anti-C5 antibody or C5-binding fragment thereof, and wherein the anti-C5 antibody or C5-binding fragment thereof inhibits cleavage of C5 into fragments C5a and C5b.

5. Applicant's IDSs, filed 5/13/2009, 1/20/2010, 9/29/2010 and 5/10/2010, are acknowledged. The foreign patent to Gotze EP0411306A1 listed on the information disclosure statement filed on 5/13/2009 fails to comply with 37 CFR 1.98(a)(3) because it does not include either a translation or a concise explanation of the relevance, as it is presently understood by the individual designated in 37 CFR 1.56(c) most knowledgeable about the content of the information. Applicant is invited to provide a copy of the English translation or a statement of relevance.

The Listed U.S. Patent to Bell et al. (2005/0191298) listed on the information disclosure statement filed on 5/13/2009 does not contain the correct Patent document number.

The reference has been considered by the examiner and the correct patent document number listed on the attached Form 892.

.

The non-patent literature document, Hillmen et al., listed on the information disclosure statement filed 9/29/2010 fails to comply with 37 CFR 1.98(a)(2), which requires a legible copy of the non-patent literature publication or that portion which caused it to be listed; and all other information or that portion which caused it to be listed. Applicant is invited to provide a copy of the reference to the examiner.

6. The first line of the specification should be amended to reflect that the current application is a national stage application of PCT/US07/06606 which claims priority to US provisional application 60/783/070.

7. 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

8. Claims 93, 95-97, and 99-100 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al., (N Engl J Med 2004; 350: 552-9, IDS Ref of 5/13/09).

Hillmen et al. teaches a pharmaceutical composition comprising eculizumab (anti-C5 monoclonal antibody) which was administered to patients with PNH at 600 mg every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12. Hillmen et al. further discloses that the anti-C5 antibody binds specifically to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶)

Hillmen et al. differs from the claimed invention by not expressly reciting a single unit dosage form of 300 mg of the anti-C5 antibody, that the single unit dosage form has a volume of 30 mL and that the single unit dosage form comprises a 10 mg/mL solution of the anti-C5 antibody.

While the prior art reference is silent with respect to the limitations that the pharmaceutical composition comprise a single unit dosage form of 300 mg, that the single unit dosage form has a volume of 30 mL (claim 96) and comprises a 10 mg/mL solution of the anti-C5 antibody or C5-binding fragment thereof (claim 97), it has been held that where the general conditions of a claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art. In re Aller, 220 F2d 454, 456, 105 USPQ 233; 235 (CCPA 1955). See MPE §§2144.05 part IIA.

In the instant case, given that Hillmen et al. teaches administration of a 600 mg and 900 mg doses which can both be obtained simply by administering two or three separate single unit dosage forms of 300 mg of the instant claimed invention, respectively, the claimed dosage forms are simply a matter of convenience based upon the needs of the patient and involves only routine skill in the art. Accordingly, it would have been obvious to one of ordinary skill in the art at the time Applicants' invention was made to supply the anti-C5 antibody or C5-binding fragment thereof in a composition comprising 300 mg single unit dosages as well as said dosages in a volume of 30 mL (i.e., 10 mg/mL).

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

9. Claims 101-102 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al., (N Engl J Med 2004; 350: 552-9, IDS Ref of 5/13/09), as applied to claims 93, 95-97 and 99-100 above, in view of Kaplan (Curr. Opin. Investig. Drugs, 3(7): 1017-23 (2002), IDS Ref of 5/13/09).

The teachings of Hillmen et al. have been discussed supra.

Hillmen et al. differs from the claimed invention by not reciting a C5-binding fragment which is pexelizumab.

Kaplan teaches the humanized single chain anti-C5 monoclonal antibody fragment, pexeizumab (p. 1018, 4th ¶) which was designed to have the advantage of enhanced tissue penetration.

Those of skill in the art would have had reason to use the anti-C5 binding fragment pexelizumab as a substitute for the anti-C5 antibody eculizumab in the administration to patients with PNH as taught by Hillmen et al. because Kaplan teaches that the antibody fragment has the advantage of enhanced tissue penetration. Substituting a known

element for another, to yield the known result, is obvious. See KSR, 550 U.S. at 416, 421.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

10. Claim 94 is rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al., (N Engl J Med 2004; 350: 552-9, IDS Ref of 5/13/09), as applied to claims 93, 95-97 and 99-100 above, and further in view of Tang et al. (Pharmaceutical Research, 21(2) (2004)).

The teachings of Hillmen et al. have been discussed supra.

Hillmen et al. differs from the claimed invention by not reciting that the pharmaceutical composition is lyophilized (claim 94).

Tang et al. teaches that lyophilization, also known as "freeze drying", is widely used for pharmaceuticals to improve the stability and long term storage stability of labile drugs, especially protein drugs (p. 191, 1st ¶).

It would have been obvious to one of ordinary skill in the art at the time Applicants' invention was made to combine the lyophilization procedure taught by Tang et al. with the anti-C5 antibody composition taught by Hillmen et al. One skilled in the art would be motivated to provide the single unit dosage form of anti-C5 antibody or a C5 binding fragment thereof in lyophilized form because Tang et al. teaches that lyophilization is commonly used to improve the stability and long term storage stability of labile drugs, especially protein drugs.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

11. Claim 98 is rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al., (N Engl J Med 2004; 350: 552-9, IDS Ref of 5/13/09), as applied to claims 93, 95-97 and 99-100 above, and further in view of Garcia et al. (Biologicals (2002) 30, 143-151).

The teachings of Hillmen et al. have been discussed supra.

Hillmen et al. differs from the claimed invention by not reciting that the pharmaceutical composition is a preservative free formulation (claim 98).

Garci et al. teaches that the influence of preservatives on the safety and stability of antivenoms may be involved in adverse reactions to antivenoms and is an issue that deserves careful consideration (p. 144, 2nd ¶; p. 149, last ¶).

It would have been obvious to one of ordinary skill in the art at the time Applicants' invention was made to provide the composition taught by Hillmen et al. in a preservative free formulation. One skilled in the art would be motivated to provide the composition in a preservative free formulation because Garci et al. teaches that the presence of preservatives can be an important safety issue in that they may lead to adverse reactions when present in antibody compositions such as antivenoms.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

12. No claim is allowed.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Ram Shukla** can be reached on (571) 272-0735. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

September 14, 2011

/JAMES ROGERS/
Examiner, Art Unit 1644

/Maher M. Haddad/
Primary Examiner, Art Unit 1644

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/225,040	05/13/2009	Leonard Bell	ALXN-P04-114	5538

28120 7590 06/01/2011

ROPES & GRAY LLP
IPRM - Floor 43
PRUDENTIAL TOWER
800 BOYLSTON STREET
BOSTON, MA 02199-3600

EXAMINER	
ROGERS, JAMES L	
ART UNIT	**PAPER NUMBER**
1644	

NOTIFICATION DATE	DELIVERY MODE
06/01/2011	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):

USPatentMail@ropesgray.com
USPatentMail2@ropesgray.com

Office Action Summary	Application No.	Applicant(s)
	12/225,040	BELL ET AL.
	Examiner	Art Unit
	JAMES ROGERS	1644

-- 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 *1* 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 *10 May 2011*.

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

3)☐ 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

4)☒ Claim(s) *93-108* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *93-108* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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)	4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)	5) ☐ Notice of Informal Patent Application
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.	6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's preliminary amendment, filed on May 10, 2011, is acknowledged. Claims 93-108 are pending and being acted upon presently.

Election/Restrictions

2. Restriction is required under 35 USC 121 and 372. This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

3. In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

I. Claims 93-102, drawn to a pharmaceutical composition comprising a single unit dosage form of an anti-C5 antibody or a C5-binding fragment thereof, wherein the single unit dosage form comprises 300 mg of the anti-C5 antibody or C5-binding fragment thereof, and wherein the anti-C5 antibody or C5-binding fragment thereof inhibits cleavage of C5 into fragments C5a and C5b.

II. Claims 103-108, drawn to a method for treating a patient afflicted with a hemolytic disease, the method comprising administering to the patient a pharmaceutical composition comprising a single unit dosage form of an anti-C5 antibody or a C5-binding fragment thereof, wherein the single unit dosage form comprises 300 mg of the anti-C5 antibody or C5-binding fragment thereof, and wherein the anti-C5 antibody or C5-binding fragment thereof inhibits cleavage of C5 into fragments C5a and C5b, in an amount and with a frequency effective to treat the hemolytic disease.

4. The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 132, they lack the same or corresponding special technical features for the following reasons:

The invention of Group II was found to have no special technical feature that defined the contribution over the prior art of Hillmen et al.: "Effect of Eculizumab on Hemolysis and Transfusion Requirements in Patients with Paroxysmal Nocturnal Hemoglobinuria" N Engl J Med 2004; 350: 552-9.

Hillmen et al. teaches treating patients with a hemolytic disease with infusions of eculizumab (600 mg) every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12 (Abstract).

Since Applicant's inventions do not contribute a special technical feature when viewed over the prior art they do not have a single general inventive concept and so lack unity of invention.

5. Applicant is advised that the reply to this requirement to be complete must include (i) an election of an invention to be examined even though the requirement may be traversed (37 CFR 1.143)**.**

6. The examiner has required restriction between product and process claims. Where applicant elects claims directed to the product, and the product claims are subsequently found allowable, withdrawn process claims that depend from or otherwise require all the limitations of the allowable product claim will be considered for rejoinder. All claims directed a nonelected process invention must require all the limitations of an allowable product claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product are found allowable, an otherwise proper restriction requirement between product claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product claim will not be rejoined. See MPEP § 821.04(b). Additionally, in order to retain the right to rejoinder in accordance with the above policy, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product claims. **Failure to do so may result in a loss of the right to rejoinder**. Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

May 24, 2011

/JAMES ROGERS/
Examiner, Art Unit 1644

/Maher M. Haddad/
Primary Examiner,
Art Unit 1644

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/225,040	05/13/2009	Leonard Bell	ALXN-P04-114	5538

28120 7590 04/11/2011

ROPES & GRAY LLP
IPRM - Floor 43
PRUDENTIAL TOWER
800 BOYLSTON STREET
BOSTON, MA 02199-3600

EXAMINER	
ROGERS, JAMES L	
ART UNIT	**PAPER NUMBER**
1644	

NOTIFICATION DATE	DELIVERY MODE
04/11/2011	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):

USPatentMail@ropesgray.com
USPatentMail2@ropesgray.com

Office Action Summary	Application No.	Applicant(s)	
	12/225,040	BELL ET AL.	
	Examiner	Art Unit	
	JAMES ROGERS	1644	

-- 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 1 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 *12 September 2008*.

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

3)☐ 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

4)☒ Claim(s) *1-25, 44, 68-92* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-25, 44, 68-92* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's preliminary amendment, filed on September 12, 2008, is acknowledged.

Claims 1-25, 44, and 68-92 are pending and being acted upon presently.

Species Election

2. Applicant is required under 35 US 121 (1) to elect a single disclosed species to which claims would be restricted if no generic claim is finally held to be allowable and (2) to list all claims readable thereon including those subsequently added.

A. Applicant is required to elect a particular assay for quality of life (e.g., FACIT-Fatigue score or EORTC QLQ-C30 score) such as one recited in claims 2 and 5. These species are distinct because the assays differ with respect to ingredients, method steps, and endpoints; thus each assay patentably distinct subject matter.

(i) If Applicant elects EORTC QLQ-C30 as an assay for quality of life, applicant is further required to elect a particular aspect of quality of life (e.g., global health status or insomnia) such as one recited in claims 7 and 8. These species are distinct because the pathological conditions differ in etiologies and therapeutic endpoints; thus each condition represents patentably distinct subject matter.

B. Applicant is required to elect a particular compound (e.g., CR1, K76 COOH, steroid, or antibody which blocks C5 cleavage) such as one recited in claims 9-17 and 80-85. These are distinct species because their structures and modes of action are different which, in turn, address different therapeutic endpoints.

C. Applicant is required to elect a particular type of disease (e.g., aplastic anemia, myelodysplastic syndrome, or paroxysmal nocturnal hemoglobinuria) such as one recited in claims 18, 74 and 90. These species are distinct because the pathological conditions differ in etiologies and therapeutic endpoints; thus each condition represents patentably distinct subject matter.

D. Applicant is required to elect a particular assay for health-adjusted life expectancy (e.g., Years of potential life lost or Years of healthy life) such as one recited in claim 75. These species are distinct because the pathological conditions differ in etiologies and therapeutic endpoints; thus each condition represents patentably distinct subject matter.

3. Applicant is required under 35 U.S.C. 121 to elect a single disclosed species for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable.

There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species,** including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election of the species may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species.

Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

4. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

April 4, 2011

/JAMES ROGERS/
Examiner, Art Unit 1644

/Maher M. Haddad/
Primary Examiner, Art Unit 1644

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/621,792	11/19/2009	Leonard Bell	ALXN-P05-114	3142

28120 7590 09/30/2010

ROPES & GRAY LLP
PATENT DOCKETING 39/41
ONE INTERNATIONAL PLACE
BOSTON, MA 02110-2624

EXAMINER	
GAMBEL, PHILLIP	
ART UNIT	**PAPER NUMBER**
1644	

MAIL DATE	DELIVERY MODE
09/30/2010	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)	
	12/621,792	BELL ET AL.	
	Examiner	Art Unit	
	Phillip Gambel	1644	

-- 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 *11/19/2009, 09/01/2010*.

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

3)☐ 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

4)☒ Claim(s) *72-84* is/are pending in the application.

4a) Of the above claim(s) *75* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *72-74, 76-84* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *01/21/2010*.
4) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *09/01/2010* .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's amendment, filed 11/19/2009, has been entered
Claims 1-71 and 85-171 have been canceled.

Claims 72-84 are pending.

2. This application is directed to the following species of administering a "compound" as set forth in claims 73 and 74 or as disclosed on pages 11-16 of the specification.

The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

These species of "compounds" are distinct because their structures, physicochemical properties and modes of action differ action to such an extent and require non-coextensive searches to such an extent that they are considered patentable distinct. Further, these "compounds" do not share a substantial structural feature essential to a common utility do not have common structure to a common utility. Therefore, they are separate and patentably distinct species.

Applicant is required under 35 U.S.C. § 121 to elect a single disclosed species of "compound" (e.g., *anti-C5 antibody or anti-C5a receptor antibody)* if elected herein for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. For example, claim 72 is generic.

There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

3. Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected species, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election of the species may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species.

Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

4. During a telephone conversation with Ryan Murphey on 09/01/2010, a provisional election was made with traverse to prosecute the species of "anti-C5a antibodies".

Affirmation of this election must be made by applicant in responding to this Office action.

Claims 72-74 and 76-84 are under consideration as they read on the elected species.

Claim 75 has been withdrawn from consideration as being drawn to a non-elected species.

5. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. 1.48(b) and by the fee required under 37 C.F.R. 1.17(h).

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

7. The application is required to be reviewed and all spelling, TRADEMARKS, and like errors corrected. Appropriate corrections are required.

Trademarks should be capitalized or accompanied by the ® or ™ symbol wherever they appear and be accompanied by the generic terminology. Although the use of trademarks is permissible in patent applications, the proprietary nature of the trademarks should be respected and every effort made to prevent their use in any manner, which might adversely affect their validity as trademarks.

Appropriate corrections are required

8. Claim 74 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 74 is indefinite in the recitation of "functional fragment of h5G1.1" in that while applicant's intent may be directed to "antigen-binding fragments thereof / C5-binding fragments thereof", the claims are no so limited and can read on any fragment.

For example, immunoglobulin Fc fragments comprised immunoglobulin effector function capabilities and often employed in therapeutic agents; however this does not appear to be applicant's intent for the claimed invention herein.

The claim is indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicant is invited to amend the claims to recite the appropriate "fragments thereof".

9. The following is a quotation of the first paragraph of 35 U.S.C. § 112:
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

10. Claim 74: h5G1.1.

It is apparent that h5G1.1 antibody is required to practice the claimed invention. As a required element, it must be known and readily available to the public or obtainable by a repeatable method set forth in the specification. If it is not so obtainable or available, the enablement requirements of 35 USC 112, first paragraph, may be satisfied by a deposit of the pertinent cell line / hybridoma which produce this antibody. See 37 CFR 1.801-1.809.

Given the disclosure on pages 17-18 of the instant specification and the claims of U.S. Patent 6,355,245 (1449) encompassing the instant 5G1.1 antibody produced by the hybridoma designated ATCC HB 11625; the conditions for the deposit of biological materials under 35 USC 112, first paragraph, with respect to h5G1.1 has been satisfied.

Note that Kaplan (Curr. Opin. Investig. Drugs 3: 1017-1023, 2002) (1449) indicates that 5G1.1 and 5G1.1 are synonyms (see entire document, including page box on the top of page 1017, column 2).

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

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

12. 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. §103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. § 103(c) and potential 35 U.S.C. §§ 102(f) or (g) prior art under 35 U.S.C. § 103(a).

13. Claims 72-74 and 76-84 are rejected under 35 U.S.C. § 102(a) as being anticipated by Hillmen et al. (Blood 102: 1858, 2003) (1449) (see entire document)
and in further evidence by Kaplan (Curr. Opin. Investig. Drugs 3: 1017-1023, 2002) (1449).

Hillmen et al. teach methods of treating paroxysmal nocturnal hemoglobulinuria (PNH), intravascular hemolysis and venous thrombosis, with an anti-C5 antibody (eculizumab) and in certain instances with erythropoietin as well, including improvement in biochemical / biological parameters associated with LDH and PNH type III cells (see entire document).

Eculizumab is derived from the h5G1.1 anti-C5 antibody, as evidenced by Kaplan.
Although the reference is silent about certain specific claimed limitations per se of a patient having paroxysmal nocturnal hemoglobulnuria (PNH) (e.g., see claims 76-84),
it does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure. See Bristol-Myers Squibb Company v. Ben Venue Laboratories 58 USPQ2d 1508 (CAFC 2001). "{i}t is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable". In re Woodruff, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990). The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145.

On this record, it is reasonable to conclude that the same PNH patients are being administered the active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., reticulocyte numbers and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to administer anti-C5 antibodies to treat PNH patients.

14. Claims 72-74 and 76-84 are rejected under 35 U.S.C. § 102(b) as being anticipated by Alexion Pharmaceuticals Signs Agreement with Lonza Biologics for Commercial Manufacturing (01/06/2003) (1449) (see entire document)
and in further evidence by Kaplan (Curr. Opin. Investig. Drugs 3: 1017-1023, 2002) (1449).

Alexion Pharmaceuticals Signs Agreement with Lonza Biologics For Commercial Manufacturing describes the commercial manufacture, commercialization and clinical studies of evaluating and treating patients with paroxysmal nocturnal hemoglobinuria with eculizumab, an antibody that binds C5.

Note, too, that Alexion is treating patents with coronary artery bypass graft surgery patients undergoing cardiopulmonary bypass as well as patients with acute myocardial infarction patients with pexelizumab, a single chain antibody that binds C5.

Both eculizumab and pexelizumab are derived from the h5G1.1 anti-C5 antibody, as evidenced by Kaplan.

Although the reference is silent about certain specific claimed limitations per se of a patient having paroxysmal nocturnal hemoglobinuria (PNH) (e.g., see claims 76-84),
it does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure. See Bristol-Myers Squibb Company v. Ben Venue Laboratories 58 USPQ2d 1508 (CAFC 2001). "{i}t is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable". In re Woodruff, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990). The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145.

On this record, it is reasonable to conclude that the same PNH patients are being administered the active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., platelet counts, reticulocyte counts and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to administer anti-C5 antibodies to treat PNH patients.

15. Claims 72-74 and 76-84 are rejected under 35 U.S.C. § 102(b) as being anticipated by Alexion Announces Results of Clinical Trial in Paroxysmal Nocturnal Hemoglobinuria Presented at the American Society of Hematology Annual Meeting (12/02/2002) (1449) (see entire document)
and in further evidence by Kaplan (Curr. Opin. Investig. Drugs 3: 1017-1023, 2002) (1449).

Alexion Announces Results of Clinical Trail in Paroxysmal Nocturnal Hemoglobinuria Presented at the American Society of Hematology Annual Meeting describes the use of eculizumab in the treatment of patients with paroxysmal nocturnal hemoglobinuria (PNH), showing a reduction in biochemical parameters of hemolysis, including the increase of Type III PNH red blood cells. Also, this announcement describes the complications of deficiencies of other blood cell types and inappropriate blood clotting known as thrombosis in patients with PNH.

Eculizumab is derived from the h5G1.1 anti-C5 antibody, as evidenced by Kaplan.

Although the reference is silent or not specific about certain specific claimed limitations per se of a patient having paroxysmal nocturnal hemoglobinuria (PNH) (e.g., see claims 76-84),
it does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure. See Bristol-Myers Squibb Company v. Ben Venue Laboratories 58 USPQ2d 1508 (CAFC 2001). "{i}t is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable". In re Woodruff, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990). The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145.

On this record, it is reasonable to conclude that the same PNH patients are being administered the active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., platelet counts, reticulocyte counts and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to administer anti-C5 antibodies to treat PNH patients.

16. Claims 72-74 are rejected under 35 U.S.C. § 102(e) as being anticipated by Bell (U.S. Patent No. 7,361,339) (see entire document)
and in further evidence by Kaplan (Curr. Opin. Investig. Drugs 3: 1017-1023, 2002) (1449).

Bell teaches and claims methods of treat acute myocardial infarction, including reducing the mortality of patients as well as treating patients in connection with angioplasty with anti-C5 antibodies, including h5G1.1 anti-C5 antibodies (see entire document, including Background, Summary and Detailed Description of Preferred Embodiments and Claims).
Note that Bell describes the advent of thrombolysis and angioplasty to restore blood flow and the role of complement activation in the formation of pro-thrombotic microparticles in the Background of the Invention (e.g., see column 1, paragraph 3 and column 2, paragraph 1)

Eculizumab is derived from the h5G1.1 anti-C5 antibody, as evidenced by Kaplan.

Although the reference is not specific about reducing thrombosis per se,
it does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure. See Bristol-Myers Squibb Company v. Ben Venue Laboratories 58 USPQ2d 1508 (CAFC 2001). "{i}t is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable". In re Woodruff, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990). The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145.

On this record, it is reasonable to conclude that the same patients with thrombotic conditions are being administered the active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters associated with myocardial infarction in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to administer anti-C5 antibodies to treat patients with myocardial infarction undergoing angioplasty.

17. Claims 72-74 and 76-84 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hillmen et al. (Blood 102: 1858, 2003) (1449) AND/OR Alexion Announces Results of Clinical Trial in Paroxysmal Nocturnal Hemoglobinuria Presented at the American Society of ematology Annual Meeting (12/02/2002) (1449) in view of Meyers et al. (Int. J. Hematol. 77: 125-132, 2003) and Evans et al. (U.S. Patent No. 6,355,245) and in further evidence by Kaplan (Curr. Opin. Investig. Drugs 3: 1017-1023, 2002) (1449).

Hillmen et al. (2003) teach methods of treating paroxysmal nocturnal hemoglobulinuria (PNH), which is characterized by intravascular hemolysis and venous thrombosis with an anti-C5 antibody and in certain instances with erythropoietin as well, including improvement in biochemical / biological parameters associated with LDH and PNH type III cells (see entire document).

Alexion Announces Results of Clinical Trail in Paroxysmal Nocturnal Hemoglobinuria Presented at the American Society of Hematology Annual Meeting describes the use of eculizumab in the treatment of patients with paroxysmal nocturnal hemoglobinuria (PNH), showing a reduction in biochemical parameters of hemolysis, including the increase of Type III PNH red blood cells. Also, this announcement describes the complications of deficiencies of other blood cell types and inappropriate blood clotting known as thrombosis in patients with PNH.

Eculizumab is derived from the h5G1.1 anti-C5 antibody, as evidenced by Kaplan.

With respect to the 5G1.1 antibody itself,
Evans et al. teaches making and using the h5G1.1. anti-C5 antibody and antigen-binding fragments for therapeutic utilities associated with blocking the complement hemolytic activity and C5a generation associated undesirable inflammatory conditions (see entire document, including Field of the Invention, Background of the Invention, Summary of the Invention, Background Physiology and Pathology, Description of the Preferred "Embodiments, Examples and Claims.

It does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure

Meyers et al. teach management issues in the treatment of paroxysmal nocturnal hemoglobinuria (see entire document), including the hallmarks of hemolysis and anemia (e.g., see pages 125-128) as well as the treatment of this syndrome with complement inhibitors such as anti-C5 antibodies particularly for the hemolysis of PNH (see Section 2.5, Complement Inhibitors), erythropoietin (see Section 2.6, Management of Nonhemolytic Anemia) and Thrombosis (see Section 3) and Bone Marrow Failure (e.g., see Section 4).

Given the nature of the clinical manifestations of PNH such as hemolysis and anemia, one of ordinary skill in the art at the time the invention was made would have been motivated to select anti-C5 antibodies to address and manage the clinical manifestations of PNH over time with an expectation of success in increase type II red blood cells, platelet counts and reticulocytes. One would have been motivated to combine these therapies with a reasonable expectation of success because each treats a different aspect of the hemolytic disease in the patient and could be reasonably expected to improve the prognosis of the patient more rapidly than either therapy alone. From the teachings of the references, it was apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

"The test of obviousness is not express suggestion of the claimed invention in any or all of the references but rather what the references taken collectively would suggest to those of ordinary skill in the art presumed to be familiar with them." See In re Rosselet, 146 USPQ 183, 186 (CCPA 1965).

"There is no requirement (under 35 USC 103(a)) that the prior art contain an express suggestion to combine known elements to achieve the claimed invention. Rather, the suggestion to combine may come from the prior art, as filtered through the knowledge of one skilled in the art." Motorola, Inc. v. Interdiqital Tech. Corp., 43 USPQ2d 1481, 1489 (Fed. Cir. 1997).

An obviousness determination is not the result of a rigid formula disassociated from the consideration of the facts of a case. Indeed, the common sense of those skilled in the art demonstrates why some combinations would have been obvious where others would not. See KSR Int'l Co. v. Teleflex Inc., 82 USPQ2d 1385 (U.S. 2007) ("The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.").

18. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., In re Berg, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); In re Goodman, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); In re Van Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and In re Thorington, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

19. Claims 72-74 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-23 of U.S. Patent No. 7,361,339.

Given that thrombosis is characteristic of treating patients with myocardial infarction undergoing angioplasty (e.g., see Background of U.S. Patent No. 7,361,339),
the patented claims of treating acute myocardial infarction, including reducing the mortality of patients as well as treating patients in connection with angioplasty with anti-C5 antibodies, including h5G1.1 anti-C5 antibodies, anticipate the instant claims.

20. Claims 72-74 are directed to an invention not patentably distinct from claims 1-23 of commonly assigned U.S. Patent No. 7,361,339 for the reasons above in Section 19.

The U.S. Patent and Trademark Office normally will not institute an interference between applications or a patent and an application of common ownership (see MPEP Chapter 2300). Commonly assigned U.S. Patent No., discussed above, would form the basis for a rejection of the noted claims under 35 U.S.C. 103(a) if the commonly assigned case qualifies as prior art under 35 U.S.C. 102(e), (f) or (g) and the conflicting inventions were not commonly owned at the time the invention in this application was made. In order for the examiner to resolve this issue, the assignee can, under 35 U.S.C. 103(c) and 37 CFR 1.78(c), either show that the conflicting inventions were commonly owned at the time the invention in this application was made, or name the prior inventor of the conflicting subject matter.
A showing that the inventions were commonly owned at the time the invention in this application was made will preclude a rejection under 35 U.S.C. 103(a) based upon the commonly assigned case as a reference under 35 U.S.C. 102(f) or (g), or 35 U.S.C. 102(e) for applications pending on or after December 10, 2004.

21. Claims 72-74 and 76-84 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-7, 9-48, 50-89, 91-129, 130, 132-163 and 168-191 of USSN 11/595,118.

The instant and copending claims are drawn to the same or nearly the same therapeutic endpoints of treating / reducing thrombosis encompassing the treatment of paroxysmal nocturnal hemoglobinuria (PNH) with the same anti-C5 antibodies to achieve the same or nearly same effects on hemopoietic elements.

While the copending claims include combination therapy, one of ordinary skill in the art would have employed conventional therapy to treat the same patients with thrombosis or PNH at the time the invention was made. Such combination therapy would anticipate the instant claims.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

22. Given copending applications encompassing similar subject matter and the restriction set forth therein,
applicant is invited to clarify which applications should be subject to rejections under the judicially created doctrine of obviousness-type double patenting.

23. No claim is allowed.

24. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (571) 272-0844. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735.

The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Phillip Gambel/

Primary Examiner
Technology Center 1600
Art Unit 1644
September 20, 2010

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/902,617	10/12/2010	Leonard Bell	ALXN-P06-114	2376

28120 7590 06/23/2011

ROPES & GRAY LLP
IPRM - Floor 43
PRUDENTIAL TOWER
800 BOYLSTON STREET
BOSTON, MA 02199-3600

EXAMINER	
GAMBEL, PHILLIP	
ART UNIT	**PAPER NUMBER**
1644	

NOTIFICATION DATE	DELIVERY MODE
06/23/2011	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):

USPatentMail@ropesgray.com
USPatentMail2@ropesgray.com

Office Action Summary	Application No.	Applicant(s)
	12/902,617	BELL ET AL.
	Examiner	Art Unit
	PHILLIP GAMBEL	1644

-- 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 *04 May 2011*.

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

3)☐ 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

4)☒ Claim(s) *131-145* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *131-145* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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)	4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)	5) ☐ Notice of Informal Patent Application
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *05/04/2011*.	6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election of the species (B) (methods of administering anti-C5 antibody and one or more additional compounds that increase hematopoiesis) and the species erythropoietin with traverse in the Response to the Election of Species Requirement, filed 05/04/2011, is acknowledged.

The traversal is on the grounds the Groups (A) and (B) are closely related and both species rely upon the recitation of "comprising" and, in turn, that searching would not imnpose an undue or substantial burden since the search would overlap.

The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

The following is noted from the Election of Species, mailed 04/04/2011.

These species of (A) and (B) are distinct because they rely upon different ingredients, methods steps and endpoints.
In turn, the steps rely upon ingredients and compounds that differ in their structures, physicochemical properties and modes of action that differ action to such an extent and require non-coextensive searches to such an extent that they are considered patentable distinct. Further, these ingredients and compounds do not share a substantial structural feature essential to a common utility do not have common structure to a common utility. Therefore, they are separate and patentably distinct species

These species of compounds are distinct because their structures, physicochemical properties and modes of action differ action to such an extent and require non-coextensive searches to such an extent that they are considered patentable distinct. Further, these compounds do not share a substantial structural feature essential to a common utility do not have common structure to a common utility. Therefore, they are separate and patentably distinct species.

Applicant is required under 35 U.S.C. § 121 to elect a single disclosed species *(e.g., A or B and if B, then specific one or more compounds)* for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 131, 136 and 141 are generic, for example.
There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

This, applicant has not done.

The election of species is maintained for the reasons of record, which are reiterated herein for applicant's convenience.

Claims 131-145 are pending.

Claims 1-130 have been canceled previously.

Claims 131-145 are under consideration in the instant application as they read on the elected invention / species, including the combination of anti-C5 antibodies and erythropoietin.

3. Applicant should update the status of the priority documents on page 1 of the specification.

USSN 11/050,543 is now abandoned.

4. The application is required to be reviewed and all spelling, TRADEMARKS, and like errors corrected. Appropriate corrections are required.

Trademarks should be capitalized or accompanied by the ® or ™ symbol wherever they appear and be accompanied by the generic terminology. Although the use of trademarks is permissible in patent applications, the proprietary nature of the trademarks should be respected and every effort made to prevent their use in any manner, which might adversely affect their validity as trademarks.

The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP 608.01.
For example, see page 40 of the specification.

Appropriate corrections are required

5. Claims 132, 137 and 142 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 132, 137 and 142 are indefinite in the recitation of "h5G1.1" because its characteristics are not known. The use of "h5G1.1" as the sole means of identifying the claimed antibody renders the claims indefinite because it is merely 1 laboratory designation which does not clearly define the claimed product, since different laboratories may use the same laboratory designation to define completely distinct antibodies or biological materials.

Amending the claims to recite the appropriate ATCC Accession Numbers would obviate this rejection.

Providing the entire amino acid sequence the "h5G1.1" antibody would obviate this rejection.

Alternatively, applicant is invited to make the record clear whether satisfaction of the requirements under 35 USC 112, second paragraph, for this particular biological material has been satisfied in current U.S. Patent 6,355,245 (1449), as described on page 19 of the specification in order to make the record of the instant application complete.

Note that if applicant is relying upon the sequence of h5G1.1, it is the entire amino acid sequence that encodes h5G1.1. that is required.

Applicant is reminded that the amendment must point to a basis in the specification so as not to add any new matter. See MPEP 714.02 and 2163.06

6. The following is a quotation of the first paragraph of 35 U.S.C. § 112:
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

7. Claims 131-145 are rejected under 35 U.S.C. § 112, first paragraph, as the specification does not contain a written description of the claimed invention, in that the disclosure does not reasonably convey to one skilled in the relevant art that the inventor(s) had possession of the claimed invention at the time the application was filed.
The specification as originally filed does not provide support for the invention as now claimed:

The specification as originally filed does not provide support for the invention as now claimed:
"with a frequency effective to maintain a serum concentration of the antibody of about 35 μg/ml to thereby maintain serum complement activity in the patient at a level below 20% of the normal serum complement activity" and
"dosing schedule … at least once every 12 days".

Applicant's amendment, filed 10/12/2010, directs support for the newly added claims to page 19, lines 6-16; page 33, lines 20-23; page 36, lines 16 – page 37, line 9 and page 40, lines 1-7 and asserts no new matter has been filed.

However, with respect to the recitation of "with a frequency effective to maintain a serum concentration of the antibody of about 35 μg/ml to thereby maintain serum complement activity in the patient at a level below 20% of the normal serum complement activity",

applicant has relied upon the particulars of measuring the capacity of patient serum samples to lyse chicken erythrocytes in measuring serum hemolytic activity under certain conditions upon a particular dosage regimen(s) (e.g., see pages 39-40 of the specification).

In contrast, the claimed "frequency" is broader than that relied upon in the description of the specification as-filed.

In contrast, the claimed serum concentration is based upon any serum complement activity under any in vivo or in vitro conditions.

However, with respect to the recitation of "dosing schedule ... at least once every 12 days",
applicant has relied upon the particulars of "a reduction in the dosing interval from 14 to 12 days to maintain levels of eculizumab about 35 μg/ml and effectively and consistently blocked serum hemolytic activity for the remainder of the extension study for both patients" (E.g., see page 37, lines 2-9 of the specification),
in which the change in dosing to 12 days was limited to the particulars of the two patients in the study,
wherein the switch to 12 days was subsequent to a particular dosing regimen and for a fixed period of time.

In contrast, the claimed dosing regimen encompasses a broader dosing range of "at least once every 12 days", which includes a 12 day regimen and more than once during a 12 day regimen,
as well as a serum activity endpoints, which has addressed above.
The claims have broaden the claimed functional readout of the dosing regimen as well as the claimed dosing regimens from that described in the specification as-filed.

The specification does not provide sufficient blazemarks nor direction for the instant methods encompassing the above-mentioned "limitations" as currently recited. The instant claims now recite limitations which were not clearly disclosed in the specification as-filed, and now change the scope of the instant disclosure as-filed. Such limitations recited in the present claims, which did not appear in the specification, as filed, introduce new concepts and violate the description requirement of the first paragraph of 35 U.S.C. 112.

Applicant is required to cancel the new matter in the response to this Office Action.

Alternatively, applicant is invited to provide sufficient written support for the "limitations" indicated above.
See MPEP 714.02 and 2163.06.

8. Claims 132, 137 and 142 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled

in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention

It is apparent that the h5G1.1 antibody is required to practice the claimed invention. As a required element, it must be known and readily available to the public or obtainable by a repeatable method set forth in the specification. If it is not so obtainable or available, the enablement requirements of 35 USC 112, first paragraph, may be satisfied by a deposit of the appropriate cell line / hybridoma which produce this antibody. See 37 CFR 1.801-1.809.

In addition to the conditions under the Budapest Treaty, applicant is required to satisfy that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent in U.S. patent applications.

Amendment of the specification to recite the date of deposit and the complete name and address of the depository is required. As an additional means for completing the record, applicant may submit a copy of the contract with the depository for deposit and maintenance of each deposit.

If the original deposit is made after the effective filing date of an application for patent, the applicant should promptly submit a verified statement from a person in a position to corroborate the fact, and should state, that the biological material which is deposited is a biological material specifically identified in the application as filed, except if the person is an attorney or agent registered to practice before the Office, in which the case the statement need not be verified. See MPEP 1.804(b).

Alternatively, applicant is invited to make the record clear whether satisfaction of the requirements under 35 USC 112, first paragraph, enablement for biological materials has been satisfied in current U.S. Patent 6,355,245 (1449), as described on page 19 of the specification in order to make the record of the instant application complete.

Note that if applicant is relying upon the sequence of h5G1.1, it is the entire amino acid sequence that encodes h5G1.1. that is required.

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

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

10. 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. §103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. § 103(c) and potential 35 U.S.C. §§ 102(f) or (g) prior art under 35 U.S.C. § 103(a).

11. Claims 131-140 are rejected under 35 U.S.C. § 102(a)(b) as being anticipated by Hillmen et al. (Blood 102: 1858, 2003) (1449) (see entire document).

Since the public availability of this document is not clear and given that certain claims are subject to a rejection under 35 USC 112, first paragraph, written description / new matter; this rejection is being applied under 35 U.S.C. § 102(a)(b).

Hillmen et al. teach methods of treating paroxysmal nocturnal hemoglobulinuria (PNH), intravascular hemolysis and venous thrombosis, with 900 mg eculizumab by i.v. infusion every other week for one year an anti-C5 antibody and completing 11 and 16 months of therapy and in certain instances with erythropoietin as well, including improvement in biochemical / biological parameters associated with LDH, PNH type III cells and complement activity (see entire document).

It does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure

Although the reference is silent about certain specific claimed limitations per se of a patient having paroxysmal nocturnal hemoglobulnuria (PNH) (e.g., see claims 133 and 138) or effective amounts to maintain serum complement activity at a certain range (e.g., see claims 131 and 136),
it does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure. See Bristol-Myers Squibb Company v. Ben Venue Laboratories 58 USPQ2d 1508 (CAFC 2001). "{i}t is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable". In re Woodruff, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990). The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145.

Given the prior art teaching of treating with 900 mg of eculizumab (tradename for h5G1.1 antibody) every other week,
the prior art teaches effective amounts of treatment with the h5G1.1 anti-C5 antibody occurs in a dosing regimen at least once every 12 days.

On this record, it is reasonable to conclude that the same PNH patients are being administered the same active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., LDH, complement activity and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,
it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to administer anti-C5 antibodies to treat PNH patients.

12. Claims 131-133, 136-139 and 141-143 are rejected under 35 U.S.C. § 102(b) as being anticipated by Hillmen (44th ASH Annual Meeting Abstract, Blood, Vol. 100, 2002) (1449) (see entire document).

Hillmen (2002) teaches that paroxysmal nocturnal hemoglubinuria (PNH) is characterized by intravascular hemolysis and venous thrombosis. Hillmen teaches that the hemolysis is complement-mediated and due to the deficiency of complement regulatory proteins on red cells, particularly the terminal complement inhibitor CD59 (see entire document). Hillmen teaches treating transfusion-dependent PNH patients with 600 mg weekly for four (4) dosea and 900 mg every other week of ECULIZUMAB, a humanized anti-C5 antibody (see entire publication). Hillmen teaches that the biological parameters of hemolysis dramatically improved or normalized for all patients within a week of starting therapy. Hillmen further teaches that mean LDH decreased greater than 50%, from 4494 +/- 3380 units/liter to 636 +/- 166 units/liter.

Although the reference is silent about certain specific claimed limitations per se of a patient having paroxysmal nocturnal hemoglobulnuria (PNH) (e.g., see claims 133, 138 and 143) or effective amounts to maintain serum complement activity at a certain range (e.g., see claims 131, 136 and 141),

it does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure. See Bristol-Myers Squibb Company v. Ben Venue Laboratories 58 USPQ2d 1508 (CAFC 2001). "{i}t is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable". In re Woodruff, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990). The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145.

Given the prior art teaching of treating with 900 mg of eculizumab (tradename for h5G1.1 antibody) every other week,

the prior art teaches effective amounts of treatment with the h5G1.1 anti-C5 antibody occurs in a dosing regimen at least once every 12 days.

On this record, it is reasonable to conclude that the same PNH patients are being administered the same active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., LDH, complement activity and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,

it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to administer anti-C5 antibodies to treat PNH patients.

13. Claims 131-145 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hillmen et al. (Blood 102: 1858, 2003) (1449) AND/OR Hillmen (44th ASH Annual Meeting Abstract, Blood, Vol. 100, 2002) (1449) in view of Meyers et al. (Int. J. Hematol. 77: 125-132, 2003) (1449) and as evidenced by Garba et al. (Singapore Med. J. 46(11):632-634, 2005) (1449) with respect to normal LDH levels.

Hillmen et al. teach methods of treating paroxysmal nocturnal hemoglobulinuria (PNH), intravascular hemolysis and venous thrombosis, with 900 mg eculizumab by i.v. infusion every other week for one year an anti-C5 antibody and completing 11 and 16 months of therapy and in certain instances with erythropoietin as well, including improvement in biochemical / biological parameters associated with LDH, PNH type III cells and complement activity (see entire document).

Hillmen (2002) teaches that paroxysmal nocturnal hemoglubinuria (PNH) is characterized by intravascular hemolysis and venous thrombosis. Hillmen teaches that the hemolysis is complement-mediated and due to the deficiency of complement regulatory proteins on red cells, particularly the terminal complement inhibitor CD59 (see entire document). Hillmen teaches treating transfusion-dependent PNH patients with 600 mg weekly for four (4) dosea and 900 mg every other week of ECULIZUMAB, a humanized anti-C5 antibody (see entire publication). Hillmen teaches that the biological parameters of hemolysis dramatically improved or normalized for all patients within a week of starting therapy. Hillmen further teaches that mean LDH decreased greater than 50%, from 4494 +/- 3380 units/liter to 636 +/- 166 units/liter.
Hillmen (2002) does not teach treating a subject with EPO.

It does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure

Given the prior art teachings of treating with 900 mg of eculizumab (tradename for h5G1.1 antibody) every other week,
the prior art teaches effective amounts of treatment with the h5G1.1 anti-C5 antibody occurs in a dosing regimen at least once every 12 days.

On this record, it is reasonable to conclude that the same PNH patients are being administered the same active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters

(e.g., LDH, complement activity and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,
it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Futher, the mean level of LDH in the PNH patients was more than 1.5 times the upper limit of a normal LDH level in a person, as evidenced by Garba, who discloses that normal LDH in a person is 247.10 +/- 19.0 units/liter (Abstract in particular). The prior art teaching clearly anticipates the claimed invention.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,
it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Meyers et al. teach management issues in the treatment of paroxysmal nocturnal hemoglobinuria (see entire document), including the hallmarks of hemolysis and anemia (e.g., see pages 125-128) as well as the treatment of this syndrome with complement inhibitors such as anti-C5 antibodies particularly for the hemolysis of PNH (see Section 2.5, Complement Inhibitors) and erythropoietin (see Section 2.6, Management of Nonhemolytic Anemia).

In addition, as to the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations",
treating PNH patients characterized by the criteria of the proportion of type III red blood cells, platelet counts, type III granulocyte clone and serum complement activity are result effective variables of controlling hemolysis and reducing the risk of venous thrombosis with anti-C5 antibodies in the treatment of PNH patient populations who are characterized by intravascular hemolyisis, venous thrombosis and aplastic anemia.

It is well settled that "discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art." In re Boesch, 617 F.2d 272, 276, 205 USPQ 215, 219 (CCPA 1980). See also Merck & Co. v. Biocraft Labs. Inc., 874 F.2d 804, 809, 10 USPQ2d

1843, 1847-48 (Fed. Cir. 1989) (determination of suitable dosage amounts in diuretic compositions considered a matter of routine experimentation and therefore obvious).

As the nature of the dosing and therapeutic endpoints were known to the ordinary artisan, it would have been obvious to optimize the frequency of anti-C5 antibodies in order to control hemolysis and the risk of venous thrombosis in a variety of PNH patients.

Given the nature of the clinical manifestations of PNH such as hemolysis and anemia, one of ordinary skill in the art at the time the invention was made would have been motivated to select and combine both anti-C5 antibodies and erythropoietin to address and manage the clinical manifestations of PNH over time. One would have been motivated to combine these therapies with a reasonable expectation of success because each treats a different aspect of the hemolytic disease in the patient and could be reasonably expected to improve the prognosis of the patient more rapidly than either therapy alone. From the teachings of the references, it was apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

"The test of obviousness is not express suggestion of the claimed invention in any or all of the references but rather what the references taken collectively would suggest to those of ordinary skill in the art presumed to be familiar with them." See In re Rosselet, 146 USPQ 183, 186 (CCPA 1965).

"There is no requirement (under 35 USC 103(a)) that the prior art contain an express suggestion to combine known elements to achieve the claimed invention. Rather, the suggestion to combine may come from the prior art, as filtered through the knowledge of one skilled in the art." Motorola, Inc. v. Interdigital Tech. Corp., 43 USPQ2d 1481, 1489 (Fed. Cir. 1997).

An obviousness determination is not the result of a rigid formula disassociated from the consideration of the facts of a case. Indeed, the common sense of those skilled in the art demonstrates why some combinations would have been obvious where others would not. See KSR Int'l Co. v. Teleflex Inc., 82 USPQ2d 1385 (U.S. 2007) ("The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.").

14. Given copending applications encompassing similar subject matter and the restriction set forth herein, applicant is invited to clarify which applications should be subject to rejections under the judicially created doctrine of obviousness-type double patenting.

15. No claim is allowed.

16. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (571) 272-0844. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735.

The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Phillip Gambel/

Primary Examiner
Technology Center 1600
Art Unit 1644
June 16, 2011

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/902,617	10/12/2010	Leonard Bell	ALXN-P06-114	2376

28120 7590 04/04/2011

ROPES & GRAY LLP
IPRM - Floor 43
PRUDENTIAL TOWER
800 BOYLSTON STREET
BOSTON, MA 02199-3600

EXAMINER	
GAMBEL, PHILLIP	
ART UNIT	**PAPER NUMBER**
1644	

NOTIFICATION DATE	DELIVERY MODE
04/04/2011	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):

USPatentMail@ropesgray.com
USPatentMail2@ropesgray.com

Office Action Summary	Application No.	Applicant(s)	
	12/902,617	BELL ET AL.	
	Examiner	Art Unit	
	PHILLIP GAMBEL	1644	

-- 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 1 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 *12 October 2010*.

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

3)☐ 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

4)☒ Claim(s) *131-145* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *131-145* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ 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).

11)☐ 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

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) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's amendment, filed 10/12/2010, has been entered.
Claims 1-130 have been canceled.
Claims 131-145 have been added.

Claims 131-145 are pending.

2. Applicant is required to elect
A) whether the claimed method do NOT comprise further administering one or more compounds that increase hematopoiesis OR
B) whether the claimed method do comprise further administering one or more compounds that increase hematopoiesis.

If applicant elects (B),
then applicant is required to elect a specific one or more compound that increase hematopoiesis from those recited in claims 135, 140 and 145 and more particularly from pages 8 and 12 of the instant specification.
If applicant elects *"one or more compounds"*, then applicant should clearly define the one or more compounds (e.g., cyclosporine, ATG, EPO derivatives, etc.).

The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

These species of (A) and (B) are distinct because they rely upon different ingredients, methods steps and endpoints.
In turn, the steps rely upon ingredients and compounds that differ in their structures, physicochemical properties and modes of action that differ action to such an extent and require non-coextensive searches to such an extent that they are considered patentable distinct. Further, these indgredients and compounds do not share a substantial structural feature essential to a common utility do not have common structure to a common utility. Therefore, they are separate and patentably distinct species

These species of compounds are distinct because their structures, physicochemical properties and modes of action differ action to such an extent and require non-coextensive searches to such an extent that they are considered patentable distinct. Further, these compounds do not share a substantial structural feature essential to a common utility do not have common structure to a common utility. Therefore, they are separate and patentably distinct species.

Applicant is required under 35 U.S.C. § 121 to elect a single disclosed species (e.g., A or B and if B, then specific one or more compounds) for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 131, 136 and 141 are generic, for example.

There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

3. Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected species, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election of the species may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species.

Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

4. Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

5. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (571) 272-0844. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735.

The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Phillip Gambel/
Primary Examiner
Technology Center 1600
Art Unit 1644
March 26, 2011

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.
13/426,973	03/22/2012	Leonard Bell	ALXN-P08-114	8851

28120 7590 01/27/2014

ROPES & GRAY LLP
IPRM - Floor 43
PRUDENTIAL TOWER
800 BOYLSTON STREET
BOSTON, MA 02199-3600

EXAMINER	
ROGERS, JAMES L	

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USPatentMail@ropesgray.com
USPatentMail2@ropesgray.com

Office Action Summary	Application No. 13/426,973	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	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* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

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

Status

1)☒ Responsive to communication(s) filed on *10/23/2013*.

☐ 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) *91,98,100-106,110-113 and 115* 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) *91,98,100-106,110-113 and 115* 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/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *05/14/2013, 10/23/2013, and 01/08/2014*.

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

4) ☐ Other: _____.

RESPONSE TO APPLICANT'S AMENDMENT

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

2. Claims 91, 98, 100-106, 110-113 and 115 are pending.

3. Applicant's IDSs, filed 05/14/2013, 10/23/2013, and 01/08/2014, are acknowledged. The references on the two IDSs submitted on 05/14/2013 have been striked-through because the references are duplicates of the references listed on the IDS, also filed on 05/14/2013 and previously considered in the prior 05/31/2013 OA.

In view of the Response filed 10/23/2013, the following rejections are maintained.

Response to Arguments

4. 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 even 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

5. Claims 91, 98, 100-106, and 112-113 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 552-9, of record in the prior 08/20/2012 office action) and Hill et al. (Haematologica 2005, of record in the prior 08/20/2012 office action), in view of Evans et al. (US 6,355,245 B1, issued 3/12/2002) and Wang (US 2005/0271660), as is evidenced by the current specification at p. 16, lines 20-25.

Applicant's arguments filed 10/23/2013 were fully considered but were not considered persuasive.

Applicant asserts that the genus of potential dosage forms for eculizumab vary widely in volume, concentration and quantity whereas the instant claims are drawn to a distinct species in which the volume, concentration, and quantity are defined. Applicant further asserts that the examiner has relied nearly exclusively on general knowledge to supply the requisite reasons for the obviousness finding which is, in essence, “convenience” (p. 3 of the response). Applicant further asserts that weekly or biweekly injections of the 154, 210 or 350 mg doses of eculizumab would require the use of multiple 300 mg vials of the instantly claimed formulation or multiple uses of the same via which would be contrary to best practices for safe injection (p. 4 of the response).

This argument was not considered persuasive because while the examiner acknowledges that the ‘245 patent does not recite the specific species claimed by applicant (i.e., a single unit dosage form comprising 300 mg of the anti-C5 antibody, wherein the single unit dosage form comprises a 10 mg/mL solution of the anti-C5 antibody in a volume of 30 mL), the ‘245 patent does teach low doses of eculizumab which is the anti-C5 antibody are therapeutically effective (see p. 5 of the prior OA). The primary art reference Hill also teaches treating the same patient population afflicted with the same disease which is PNH by administering the same anti-C5 antibody, albeit at a higher dose of 600 mg (see p.3 of the prior OA). Those of skill in the art would look to the ‘245 patent for guidance in arriving at lower dosage amounts such as a single unit 300 mg or 10 mg/ml solution wherein the single unit dosage form has a volume of 30 mL as currently recited by applicant because the '245 teaches that lower dosages are also therapeutically effective. Where the general conditions of a claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art. In re Aller, 220 F2d 454, 456, 105 USPQ 233; 235 (CCPA 1955). See MPE §§2144.05 part IIA. It remains the examiners position that given the teachings of Hill with respect to treating the exact same patient population and the same disease with the same antibody, that arriving at applicants' dosage would have amounted to no more than routine skill in the art in light of the ‘245 patent teachings that eculizumab is also effective at lower concentrations than the 600 mg dose taught by Hill.

In short, applicant is attempting to remove from the public domain methods of treating a specific disease with a specific therapeutic at a specific dose. The only difference between what is now claimed and what was known in the prior art is the single unit dosage size recited in the claims. The same therapeutic (eculizumab) was already known to be effective for treating the same disease (PNH). While the reference by Hillmen discloses methods of administering higher doses than are now claimed, Hill indicates that it would be effective at a range of doses including those now claimed. Applicant is requesting a 20-year right to exclude others from practicing the invention as claimed, on the theory that putting the therapeutic antibody in 30 ml at 10 mg/ml represents a non-obvious contribution over the art. The examiner is of the position that this difference could have easily been arrived at by one of ordinary skill in the art and does not merit a 20-year right to exclude others.

6. Claims 91, 98, 100-106, 110 and 112-113 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 552-9, of record in the prior 08/20/2012 office action) and Hill et al. (Haematologica 2005, of record in the prior 08/20/2012 office action), in view of Evans et al. (US 6,355,245 B1, issued 3/12/2002) and Wang (US 2005/0271660), as applied to claims 91, 98, 100-106, and 112-113 above, in further view of Garcia et al. (Biologicals (2002) 30, 143-151, of record in the prior 08/20/2012 office action).

As applicant has not made any arguments with respect to the claimed difference that the composition is preservative free as in claim 110, the rejection from the prior OA is maintained.

7. Claims 91, 98, 100-106, 111-113 and 115 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 552-9, of record in the prior 08/20/2012 office action) and Hill et al. (Haematologica 2005, of record in the prior 08/20/2012 office action), in view of Evans et al. (US 6,355,245 B1, issued 3/12/2002) and Wang (US 2005/0271660), as applied to claims 91,98, 100-106, and 112-113 above, in further view of Rosse et al. (Hematology Am Soc Hematol Edc. Program, 2004, 48-62, of record in the prior 08/20/2012 office action).

As applicant has not made any arguments with respect to the claimed difference concerning the treatment schedule set forth in claim 111, the rejection from the prior OA is maintained.

8. **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is 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 date of this final action.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

January 17, 2014

/JAMES ROGERS/
Examiner, Art Unit 1644

/DANIEL E. KOLKER/
Supervisory Patent Examiner, Art Unit 1644
January 21, 2014

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/426,973	03/22/2012	Leonard Bell	ALXN-P08-114	8851

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ROPES & GRAY LLP
IPRM - Floor 43
PRUDENTIAL TOWER
800 BOYLSTON STREET
BOSTON, MA 02199-3600

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
05/31/2013	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):

USPatentMail@ropesgray.com
USPatentMail2@ropesgray.com

Office Action Summary	Application No. 13/426,973	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	AIA (First Inventor to File) Status No

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Period for Reply

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- 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 *22 January 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) *91,98,100-106,110-113 and 115* 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) *91,98,100-106,110-113 and 115* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

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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.

Interim copies:

a)☐ All b)☐ Some c)☐ None of the: Interim copies of the priority documents have been received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *1/22/2013, 5/14/2013*.

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

4) ☐ Other: _____.

DETAILED ACTION

1. Applicant's amendment filed 01/22/2013 is acknowledged.

Claims 91, 98, 100-106, 110-113, and 115 are pending and under consideration in the instant examination as they read on a method for treating a patient that (i) is afflicted with a hemolytic disease and (ii) has been vaccinated against Neisseria meningitides, the method comprising administering to the patient an effective amount of an anti-C5 antibody that inhibits cleavage of C5 into C5a and C5b.

2. The examiner acknowledges a conversation with Anita Varma on May 14, 2013 about the possible allowance of claims 91, 98, 100-106, 110 and 112-113. Upon further consideration, however, a new rejection under 35 USC 103 is set forth infra.

3. Applicant's amendment, filed 01/22/2013, has overcome the enablement rejection under 35 USC 112, 1st paragraph and the rejections under 35 USC 102(b) which were of the previous office action mailed on 08/20/2012.

4. Applicant's IDSs, filed on 01/22/2013 and 05/14/2013, are acknowledged and have been considered.

5. Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of 35 U.S.C. 112(a) or the first paragraph of 35 U.S.C. 112 (pre-AIA). See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosure of the prior-filed application, US Provisional Application No. 60/783,070, fails to provide adequate support or enablement in the manner provided by 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph for one or more claims of this application. In particular, the claims as currently amended recite administration of "a pharmaceutical composition comprising a single unit dosage form ... wherein the single unit dosage form comprises 300 mg of the anti-C5 antibody, wherein the single unit dosage form comprises a 10 mg/mL solution of the anti-C5 antibody, wherein the single unit dosage form has a volume of 30 mL, and wherein the anti-C5 antibody is eculizumab".

Accordingly, the priority date of the instant claims of the current application is considered to be 3/15/2007, the filing date of PCT/US2007/006606.

6. 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 even 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

7. Claims 91, 98, 100-106, and 112-113 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 552-9, of record in the prior 08/20/2012 office action) and Hill et al. (Haematologica 2005, of record in the prior 08/20/2012 office action), in view of Evans et al. (US 6,355,245 B1, issued 3/12/2002) and Wang (US 2005/0271660), as is evidenced by the current specification at p. 16, lines 20-25.

With respect to claims 91, 112-113 and 115, Hillmen teaches a method for treating a patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶). Hillmen further teaches that patients were vaccinated against N. meningitides before treatment (p. 554, lines 2-4).

In a particular embodiment, Hillmen further teaches a pharmaceutical composition comprising eculizumab (anti-C5 monoclonal antibody) which was administered to patients with PNH at 600 mg every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12.

While Hillmen does not specifically recite that the patient afflicted with PNH is "due to a C3b-mediated vascular clearance of PNH erythrocytes through the reticuloendothelial system" as in claim 103, the prior art of treating the same PNH populations with the same composition (anti-C5 antibody) to achieve the same therapeutic effect does not differ from the claimed methods. The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145. As is further evidenced by Jasinski, moreover, mice studies indicate that the shortened survival of PIGA negative RBCs which causes PNH is mediated by macrophage clearance (clearance of PNH erythrocytes through the reticuloendothelial system) (abstract).

With respect to claim 104, Hillmen teaches that PNH is characterized by hemolytic anemia which renders the patient transfusion dependent and that patients continued to receive transfusion when they had symptoms of anemia (p. 554, 1st¶ under "Investigations" and p. 557, 1st ¶ under "Discussion"). Hillmen further teaches that Type III erythrocytes increased significantly but less than 100% ("from a mean of 36.7 +5.9 to 59.2+8.0 percent") (p. 556, 3rd ¶). Thus the recitation "wherein the patient is anemic and the anemia results at least in part from extravascular hemolysis, and wherein the patient remains anemic following treatment with the anti-C5 antibody" is considered to be inherently taught by Hillmen.

With respect to claim 105, Hillmen teaches that the patient population included men and women of 18 years of age and older who had received a diagnosis of PNH at least 6 months earlier and had received at least 4 red cell transfusions in the preceding 12 months (p. 553, last ¶) and that the percentage of type III erythrocytes increased from a mean of 36.7 +5.9 percent before treatment to 49.2 +8.0 percent at the end of 12 weeks of treatment (the patient is at least 18 years of age and has (i) a PNH type III erythrocyte population of greater than 10%) (p. 556, 2nd ¶).

Hill also teaches treating patients afflicted with PNH by administering eculizumab that specifically targets C5 (anti-C5 antibody), blocking the generation of C5a and the formation of C5b-9 (inhibits cleavage of C5 into fragments C5a and C5b) (abstract; p. 2559, 3rd l[) .

With respect to claim 98, Hill teaches that sustained efficacy and safety of eculizamab in a 1 -year study (anti-C5 antibody is administered to the patient for at least 6 months) (p. 2564, 1st ¶).

With respect to claims 100-102, Hill teaches a hemoglobin level of 10.0 + 0.4 prior to treatment with eculizumab (patient has a hemoglobin level less than 12-14 g/dL if a man or 10-12 g/dL is a woman).

With respect to claim 106, Hill (I) teaches a patient with a history of PNH and aplastic anemia (p. 2563, last I[).

The prior art references differ from the claimed invention in the recitation that the pharmaceutical composition of eculizumab comprises a single unit dosage form of 300 mg comprising 30 mL at 10 mg/mL.

However, the '245 patent teaches antibody 5G1.1, which is the same as eculizumab (see present specification, p. 16 lines 20-25), is effective at doses of 2.2, 3, or 5 mg per kg body weight; this corresponds to 154, 210, or 350 mg of antibody for a 70kg adult human ('245 patent, column 16 lines 10-30). '245 patent also teaches that eculizumab is therapeutically effective when as little as 210 mg are present (column 16 lines 10-15). '245 teaches that the antibodies are particularly advantageous in that they are highly active even when administered in low doses (col 8 lines 25-32), providing a reasonable expectation of success in treating PHN with a dose less than what was disclosed by Hillmen. The reference teaches that it is within the skill of an artisan, particularly a physician, to determine the optimal dose (column 18 lines 15-30). The reference teaches sterile formulations are known in the art and can include various pharmaceutical excipients, etc. (col 18 lines 30-45).

However neither Hillmen nor '245 teaches a 300 mg single unit dosage as recited in the claims.

Nevertheless it would have been obvious to one of ordinary skill in the art to modify the teachings of Hillmen and Hill to administer 300 mg single unit dosages. Hillmen teaches that both 600 and 900 mg dosages can be used, and since '245 patent teaches that 210 or 350 mg of antibody would be expected to be therapeutically effective, arriving at the 300 mg dosage would be routine, convenient and obvious. One of ordinary skill in the art would have a reasonable expectation of success, as '245 teaches that the antibody exerts its effect at low doses.

Finally, US 2005/0271660 teaches that eculizumab is highly soluble, including at up to 30 mg per ml (paragraph 0171, Figure 10). Therefore the artisan of ordinary skill would have had a reasonable expectation of success in making a 10 mg / ml solution of the antibody.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of

ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Applicant's arguments filed at pgs. 8-9 of the 01/22/2013 response have been fully considered but were not deemed persuasive.

Applicant asserts that it is well known in the art that the dose of an antibody will vary depending on such factors as the manner of administration, the particular symptoms of the patient being treated, the overall health, condition, size, and age of the patient and the judgment of the prescribing physician. Applicant further asserts that in order to produce a solution of eculizumab at 10 mg/mL, the inventors had to know that eculizumab was sufficiently soluble for a 10 mg/mL formulation.

This argument was not considered persuasive because the '245 patent teaches that optimal dosing of eculizumab is within the skill of an artisan, particularly a physician, as noted above. In addition, the '660 publication teaches that eculizumab is highly soluble, including at up to 30 mg per ml providing a reasonable expectation of success in making a 10 mg/ml solution of the antibody as is also noted above. Given the additional teachings of the '245 patent that eculizumab is effective at lower doses, it would have been obvious to a person of ordinary skill in the art at the time the invention was made to arrive at the lower single unit dosage form as currently claimed.

8. Claims 91, 98, 100-106, 110 and 112-113 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 552-9, of record in the prior 08/20/2012 office action) and Hill et al. (Haematologica 2005, of record in the prior 08/20/2012 office action), in view of Evans et al. (US 6,355,245 B1, issued 3/12/2002) and Wang (US 2005/0271660), as applied to claims 91, 98, 100-106, and 112-113 above, in further view of Garcia et al. (Biologicals (2002) 30, 143-151, of record in the prior 08/20/2012 office action).

The prior art teachings of Hillmen, Hill, the '245 patent and the '660 publication are discussed supra.

The reference teachings differ from the claimed invention by not reciting that the pharmaceutical composition is a preservative free formulation as in claim 110.

Garci et al. teaches that the influence of preservatives on the safety and stability of antivenoms may be involved in adverse reactions to antivenoms and is an issue that deserves careful consideration (p. 144, 2nd ¶; p. 149, last ¶).

It would have been obvious to one of ordinary skill in the art at the time applicant's invention was made to provide the composition taught by the primary references in a preservative free formulation. One skilled in the art would be motivated to provide the composition in a preservative free formulation because Garci teaches that the presence

of preservatives can be an important safety issue in that they may lead to adverse reactions when present in antibody compositions such as antivenoms.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

9. Claims 91, 98, 100-106, 111-113 and 115 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 552-9, of record in the prior 08/20/2012 office action) and Hill et al. (Haematologica 2005, of record in the prior 08/20/2012 office action), in view of Evans et al. (US 6,355,245 B1, issued 3/12/2002) and Wang (US 2005/0271660), as applied to claims 91, 98, 100-106, and 112-113 above, in further view of Rosse et al. (Hematology Am Soc Hematol Edc. Program, 2004, 48-62, of record in the prior 08/20/2012 office action).

The prior art teachings of Hillmen, Hill, the '245 patent and the '660 publication are discussed supra.

The reference teachings differs from the claimed invention by not reciting the specific treatment schedule set forth in claim 111, "(i) 600 mg of the antibody every 7 ±2 days for the first 4 weeks, (ii) 900 mg of the antibody for the fifth dose 7 ± days after 9i), and (iii) 900 mg of the antibody every 14 ± 2 days thereafter, wherein the administration occurs via 25 to 45 minute intravenous infusion" as in claims 111 and 115.

Nevertheless, it would have been obvious to one of ordinary skill in the art to administer eculizumab according to the claimed recited treatment schedule above because, as stated supra, Hillman teaches a pharmaceutical composition comprising eculizumab (anti-C5 monoclonal antibody) which was administered to patients with PNH at 600 mg every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12.

Rosse also teaches therapeutic treatment of patients with transfusion-dependent paroxysmal nocturnal hemoglobinuria (PNH), by administering to the patients an effective amount of eculizumab, which blocks cleavage of C5 into C5a and C5b (Fig. 4; p. 48, 1 st ¶; p. 53, 2nd column, lines 16-18).

In a specific treatment schedule, Rosse further teaches the exact same treatment regimen where eculizumab (anti-C5 antibody) was given at a dose of 600 mg every week for 4 weeks (600 mg of the antibody every 7 + 2 days for the first 4 weeks) and then at a dose of 900 mg every other week (900 mg of the antibody for the fifth dose 7 + 2 days after and 900 mg of the antibody every 14 + 2 days thereafter). Rosse further

teaches that the drug is given by intravenous infusion over 30 minutes (administration of the antibody to the patient is to occur via 25 to 45 minutes intravenous infusion).

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

10. No claim is allowed.

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

May 20, 2013

/JAMES ROGERS/
Examiner, Art Unit 1644

/DANIEL E. KOLKER/
Supervisory Patent Examiner, Art Unit 1644
May 24, 2013

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ROPES & GRAY LLP
IPRM - Floor 43
PRUDENTIAL TOWER
800 BOYLSTON STREET
BOSTON, MA 02199-3600

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
08/20/2012	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):

USPatentMail@ropesgray.com
USPatentMail2@ropesgray.com

Office Action Summary	Application No.	Applicant(s)	
	13/426,973	BELL ET AL.	
	Examiner	Art Unit	
	JAMES ROGERS	1644	

-- 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 *22 March 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) *91-115* 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) *91-115* 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 *8/13/2012*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Claims 91-115 are pending.

2. Claims 91-115 are under consideration in the instant examination as they read on a method for treating a patient that (i) is afflicted with a hemolytic disease and (ii) has been vaccinated against Neisseria meningitides, the method comprising administering to the patient an effective amount of an anti-C5 antibody that inhibits cleavage of C5 into C5a and C5b.

3. Applicant's IDS, filed 8/13/2012, is acknowledged and has been considered.

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

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

5. Claims 91-98 and 106-115 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for treatment of paroxysmal nocturnal hemoglobinuria (PNH) by administering to a patient an effective amount of an anti-C5 antibody, does not reasonably provide enablement for a method for treatment of **any hemolytic disease** by administering an anti-C5 antibody. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required to practice the claimed invention are summarized *In re Wands* (858 F2d 731,737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)). The factors most relevant to this rejection are the scope of the claim, the amount of direction or guidance provided, the lack of sufficient working examples, the unpredictability in the art and the amount of experimentation required to enable one of skill in the art to practice the claimed invention.

Claims 91-98 and 106-115 are genus claims which encompass a method for treating a patient who suffers from **any type of hemolytic disease** by administering to the patient an effective amount of an anti-C5 antibody.

While the claims encompass treating a patient suffering from any type of hemolytic disease, the specification discloses a phase III study which evaluated the effect of eculizumab (an anti-C5 antibody) on a cohort of 87 transfusion dependent patients suffering from a single type of hemolytic disease, paroxysmal nocturnal hemoglobinuria (PNH) (p. 2, 1st ¶)

As evidenced by the specification, PNH is a disease which is characterized by hemolysis due to the absence of the GPI-anchored complement regulatory protein CD59 which blocks the formation of the terminal complement complex on the cell surface (p. 2, 2nd ¶). However, other hemolytic diseases do not share the same etiology as PNH. For example, sickle cell disease, which is also characterized by intravascular hemolysis" is caused by a mutation in the β-globin chain of hemoglobin (Kato, Blood Reviews, 2007, p. 38, 1st ¶Fig. 1), and at least one study has not identified any complement abnormality in sickle cell disease (Strauss, Pediat. Res. 11(4), 1997, abstract). In addition, as is stated by Stuart et al., sickle cell disease "is a systemic disease of monumental complexity" (Lancet, 364, 2004, p. 1357, last ¶)

In re Fisher, 166 USPQ 18 indicates that the more unpredictable an area is, the more specific enablement is necessary in order to satisfy the statute. Because the current claims encompass a large number of different hemolytic diseases, each with potentially different pathophysiological mechanisms, it is not clear that reliance upon a method for treating a single type of hemolytic disease (i.e., PNH) accurately reflects the relative mammal efficacy of the claimed therapeutic strategy for any type of hemolytic disease.

Reasonable correlation must exist between the scope of the claims and scope of the enablement set forth. In view on the quantity of experimentation necessary the limited working examples, the nature of the invention, the state of the prior art, the unpredictability of the art and the breadth of the claims, it would take undue trials and errors to practice the claimed invention.

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

> A person shall be entitled to a patent unless –
>
> (b) *the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.*

7. Claims 113-115 are rejected under 35 U.S.C. 102(b) as being anticipated by Rosse et al. (hematology Am Soc Hematol Edc. Program, 2004, 48-62).

Rosse teaches therapeutic treatment of patients with transfusion-dependent paroxysmal nocturnal hemoglobinuria (PNH), which is characterized by chronic intravascular hemolysis (method for treating a patient that is afflicted with a hemolytic disease) by administering to the patients an effective amount of eculizumab, which is a humanized chimeric anti-C5 antibody (whole antibody) which blocks cleavage of C5 into C5a and C5b (Fig. 4; p. 48, 1st ¶; p. 53, 2nd column, lines 16-18).

Rosse further teaches that eculizumab (anti-C5 antibody) has a completely nonfunctional Fc domain (p. 53, lines 10-13). Because the constant region is nonfunctional, eculizumab would necessarily have decreased effector function with respect to antibody-dependent cell mediated cytotoxity (ADCC) and complement dependent cytotoxicity (CDC) relative to the corresponding native constant region.

With respect to claim 115, Rosse further teaches that eculizumab (anti-C5 antibody) was given at a dose of 600 mg every week for 4 weeks (600 mg of the antibody every 7 ± 2 days for the first 4 weeks) and then at a dose of 900 mg every other week (900 mg of the antibody for the fifth dose 7 ± 2 days after and 900 mg of the antibody every 14 ± 2 days thereafter). Rosse further teaches that the drug is given by intravenous infusion over 30 minutes (administration of the antibody to the patient is to occur via 25 to 45 minutes intravenous infusion).

The reference teachings anticipate the claimed invention.

8. Claims 91-93, 96-97, 99, 103-105, and 112-115 are rejected under 35 U.S.C. 102(b) as being anticipated by Hillmen et al. (N Engl J Med 2004; 350: 552-9), as is evidenced by Jasinski (Blood, 103, 2004, 2827-2834) and Rosse et al. (hematology Am Soc Hematol Edc. Program, 2004, 48-62).

With respect to claims 91-93, 99, and 112-115, Hillmen teaches a method for treating a patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) which is a disease characterized by hemolytic anemia (patient afflicted with a hemolytic disease) (p. 557, 2nd ¶) by administering to the patient an effective amount of eculizumab (whole antibody) (p. 552, “methods”) which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶). Hillmen further teaches that patients were vaccinated against *N. meningitides* before treatment (p. 554, lines 2-4). As is evidenced by Rosse, eculizumab (anti-C5 antibody) has a completely nonfunctional Fc domain (p. 53, lines 10-13). Because the constant region is nonfunctional, eculizumab would necessarily have decreased effector function with respect to antibody-dependent cell mediated cytotoxity (ADCC) and complement dependent cytotoxicity (CDC) relative to the corresponding native constant region.

Claim 103 is included because while Hillmen does not specifically recite that the patient afflicted with PNH is “due to a C3b-mediated vascular clearance of PNH erythrocytes through the reticuloendothelial system”, the prior art of treating the same PNH populations with the same composition (anti-C5 antibody) to achieve the same therapeutic effect does not differ from the claimed methods. The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or

obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145. As is further evidenced by Jasinski, moreover, mice studies indicate that the shortened survival of PIGA negative RBCs which causes PNH is mediated by macrophage clearance (clearance of PNH erythrocytes through the reticuloendothelial system) (abstract).

Claim 104 is included because Hillmen teaches that PNH is characterized by hemolytic anemia which renders the patient transfusion dependent and that patients continued to receive transfusion when they had symptoms of anemia (p. 554, 1st under "Investigations" and p. 557, 1st ¶ under "Discussion"). Hillmen further teaches that Type III erythrocytes increased significantly but less than 100% ("from a mean of 36.7 ±5.9 to 59.2±8.0 percent") (p. 556, 3rd ¶). Thus the recitation "wherein the patient is anemic and the anemia results at least in part from extravascular hemolysis, and wherein the patient remains anemic following treatment with the anti-C5 antibody" is considered to be inherently taught by Hillmen.

Claim 105 is included because Hillmen teaches that the patient population included men and women of 18 years of age and older who had received a diagnosis of PNH at least 6 months earlier and had received at least 4 red cell transfusions in the preceding 12 months (p. 553, last ¶) and that the percentage of type III erythrocytes increased from a mean of 36.7 ±5.9 percent before treatment to 49.2 ±8.0 percent at the end of 12 weeks of treatment (the patient is at least 18 years of age and has (i) a PNH type III erythrocyte population of greater than 10%) (p. 556, 2nd ¶).

The reference teachings anticipate the claimed invention.

9. 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

10. Claims 91-93, 96-106 and 111-115 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rosse et al. (Hematology 2004) OR Hill (Blood, 2005, hereafter "Hill (I)") OR Hill et al. ((Haematologica 2005, hereafter "Hill (II)"), each in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), as is evidenced by Jasinski (Blood, 103, 2004, 2827-2834).

With respect to claims 91-93, 99, and 112, Rosse teaches therapeutic treatment of patients with transfusion-dependent paroxysmal nocturnal hemoglobinuria (PNH), which is characterized by chronic intravascular hemolysis (method for treating a patient that is afflicted with a hemolytic disease) by administering to the patients an effective amount of eculizumab, which is a humanized chimeric anti-C5 antibody (whole antibody) which blocks cleavage of C5 into C5a and C5b (Fig. 4; p. 48, 1st ¶; p. 53, 2nd column, lines 16-18).

With respect to claims 96-97, 113 and 114, Rosse further teaches that eculizumab (anti-C5 antibody) has a completely nonfunctional Fc domain (p. 53, lines 10-13). Because the constant region is nonfunctional, eculizumab would necessarily have decreased effector function with respect to antibody-dependent cell mediated cytotoxity (ADCC) and complement dependent cytotoxicity (CDC) relative to the corresponding native constant region.

With respect to claims 111 and 115, Rosse teaches that eculizumab (anti-C5 antibody) was given at a dose of 600 mg every week for 4 weeks (600 mg of the antibody every 7 ± 2 days for the first 4 weeks) and then at a dose of 900 mg every other week (900 mg of the antibody for the fifth dose 7 ± 2 days after and 900 mg of the antibody every 14 ± 2 days thereafter). Rosse further teaches that the drug is given by intravenous infusion over 30 minutes (administration of the antibody to the patient is to occur via 25 to 45 minutes intravenous infusion).

Rosse further teaches that deficiency of complement molecules after C5 remarkably have little phenotype with the exception of an increased risk by encapsulated organisms such as *Neisseria meningitides* (p. 53, lines 1-3).

With respect to claims 91-93, Hill (I) teaches treating patients afflicted with PNH by administering eculizumab which is a humanized monoclonal antibody (whole antibody) that specifically targets C5 (anti-C5 antibody), blocking the generation of C5a and the formation of C5b-9 (inhibits cleavage of C5 into fragments C5a and C5b) (abstract; p. 2559, 3rd ¶)

With respect to claim 98, Hill (I) teaches that sustained efficacy and safety of eculizamab in a 1-year study (anti-C5 antibody is administered to the patient for at least 6 months) (p. 2564, 1st ¶).

With respect to claims 100-102, Hill (I) teaches a hemoglobin level of 10.0 ± 0.4 prior to treatment with eculizumab (patient has a hemoglobin level less than 12-14 g/dL if a man or 10-12 g/dL is a woman).

Claim 104 is included because Hill(I) teaches that the trigger for transfusion before and during the study remained unchanged for each patient and was based on the occurrence of symptoms resulting from anemia, hemolysis, or both (p. 2560 under "clinical investigations"). Thus the recitation "wherein the patient is anemic and the anemia results at least in part from extravascular hemolysis, and wherein the patient remains anemic following treatment with the anti-C5 antibody" is considered to be inherently taught by Hill(I).

With respect to claim 106, Hill (I) teaches a patient with a history of PNH and aplastic anemia (p. 2563, last ¶).

With respect to claim 99, and 101, Hill II teaches treatment of two PNH patients (patient afflicted with a hemolytic disease) by administering eculizumab (anti-C5 antibody) (abstract).

With respect to claim 105, Hill II further teaches that the first 27 year old (patient is at least 18 years of age) during the one year period prior to eculizumab treatment hematological parameters including 47% type III PNH RBCs at baseline (a PNH type II erythrocyte population of greater than 10 %) and that since being diagnosed with PNH in 1998 to treatment in July 2002, the patient was transfusion dependent receiving about 1-2 units/month (received at least 4 transfusion during the 12 months immediately preceding the first treatment with the anti-C5 antibody) (p. e111, 3rd ¶).

Claim 103 is included because while the prior art references do not specifically recite that the patient afflicted with PNH is "due to a C3b-mediated vascular clearance of PNH erythrocytes through the reticuloendothelial system", the prior art of treating the same PNH populations with the same composition (anti-C5 antibody) to achieve the same therapeutic effect does not differ from the claimed methods. The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145. As is further evidenced by Jasinski, moreover, mice studies indicate that the shortened survival of PIGA negative RBCs which causes PNH is mediated by macrophage clearance (clearance of PNH erythrocytes through the reticuloendothelial system) (abstract).

The prior art references differ from the claimed invention by the recitation that the patient has been vaccinated against Neisseria meningitides.

With respect to claims 91-93, 99, and 112, Hillmen teaches a method for treating a patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) which is a disease characterized by hemolytic anemia (patient afflicted with a hemolytic disease) (p. 557, 2nd ¶) by administering to the patient an effective amount of eculizumab (whole antibody) (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶). Hillmen further teaches that patients were vaccinated against *N. meningitides* before treatment (p. 554, lines 2-4).

It would be obvious and within the skill of one in the art at the time the invention was made to vaccinate against *Neisseria meningitides* as taught by Hillmen in a method of treating PNH by administration of an anti-C5 antibody such as eculizamab as taught by either Rosse, Hill I or Hill II.

One of ordinary skill in the art at the time the invention was made would have been motivated to because eculizamab targets C5 thereby preventing cleavage of C5, and Rosse teaches that deficiency of complement molecules after C5 result in an increased risk by encapsulated organisms such as *Neisseria meningitides* (p. 53, lines 1-3). Thus, vaccination against *N. meningitides* prior to administration of eculizamab (anti-C5 antibody) for the treatment of PNH as taught by Hillmen would be a prudent measure against the risk of infection from *N. meningitides* in any method where an anti-C5 antibody is administered to a patient.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

11. Claims 107-110 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rosse et al. (Hematology 2004) OR Hill (Blood, 2005, hereafter "Hill (I)") OR Hill et al. ((Haematologica 2005, hereafter "Hill (II)") in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), as applied to claims 91-93, 96-102, 105-106 and 111-115 above, and further in view of Garcia et al. (Biologicals (2002) 30, 143-151).

The teachings of Rosse, Hill(I), Hill(II) and Hillmen have been discussed supra.

Hillmen further teaches a pharmaceutical composition comprising eculizumab (anti-C5 monoclonal antibody) which was administered to patients with PNH at 600 mg every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12.

The prior art references differ from the claimed invention by not expressly reciting a single unit dosage form of 300 mg of the anti-C5 antibody, that the single unit dosage form has a volume of 30 mL and that the single unit dosage form comprises a 10 mg/mL solution of the anti-C5 antibody as in claims 107-109.

The reference teachings also differ from the claimed invention by not reciting that the pharmaceutical composition is a preservative free formulation as in claim 110.

Garci et al. teaches that the influence of preservatives on the safety and stability of antivenoms may be involved in adverse reactions to antivenoms and is an issue that deserves careful consideration (p. 144, 2nd ¶; p. 149, last ¶).

It would have been obvious to one of ordinary skill in the art at the time applicant's invention was made to provide the composition taught by the primary references in a preservative free formulation. One skilled in the art would be motivated to provide the composition in a preservative free formulation because Garci teaches that the presence of preservatives can be an important safety issue in that they may lead to adverse reactions when present in antibody compositions such as antivenoms.

While the prior art references are silent with respect to the limitations that the pharmaceutical composition comprise a single unit dosage form of 300 mg, that the single unit dosage form has a volume of 30 mL (claim 96) and comprises a 10 mg/mL solution of the anti-C5 antibody or C5-binding fragment thereof (claim 97), it has been held that where the general conditions of a claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art. In re Aller, 220 F2d 454, 456, 105 USPQ 233; 235 (CCPA 1955). See MPE §§2144.05 part IIA.

In the instant case, given that Hillmen teaches administration of a 600 mg and 900 mg doses which can both be obtained simply by administering two or three separate single unit dosage forms of 300 mg of the instant claimed invention, respectively, the claimed dosage forms are simply a matter of convenience based upon the needs of the patient and involves only routine skill in the art. Accordingly, it would have been obvious to one of ordinary skill in the art at the time Applicants' invention was made to supply the anti-C5 antibody or C5-binding fragment thereof in a composition comprising 300 mg single unit dosages as well as said dosages in a volume of 30 mL (i.e., 10 mg/mL).

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

12. Claims 94-95 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rosse et al. (Hematology 2004) and Hill (Blood, 2005, hereafter "Hill (I)") and Hill et al. ((Haematologica 2005, hereafter "Hill (II)") and Hillmen et al. (N Engl J Med 2004; 350: 552-9), as applied to claims 91-93, 96-102, 105-106 and 111-115 above, and further in view of Kaplan (Curr. Opin. Investig. Drugs, 3(7): 1017-23 (2002), IDS Ref of 5/13/09).

The teachings of Rosse, Hill(I), Hill (II), and Hillmen have been discussed supra.

The prior art references differ from the claimed invention by not reciting that the anti-C5 antibody is a C5-binding fragment such as an scFv as in claim 94 and that the anti-C5 antibody is pexelizumab as in claim 95.

Kaplan teaches the humanized single chain (scFv) anti-C5 monoclonal antibody fragment, pexelizumab (p. 1018, 4th ¶) which was designed to have the advantage of enhanced tissue penetration.

Those of skill in the art would have had reason to use the anti-C5 binding fragment pexelizumab as a substitute for the anti-C5 antibody eculizumab in the administration to patients with PNH as taught by the prior art references because Kaplan teaches that the antibody fragment has the advantage of enhanced tissue penetration. Substituting a known element for another, to yield the known result, is obvious. See KSR, 550 U.S. at 416, 421.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

13. No claim is allowed.

14. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you

have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

August 15, 2012

/JAMES ROGERS/
Examiner, Art Unit 1644

/Maher M. Haddad/
Primary Examiner, Art Unit 1644

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/335,518	12/22/2011	Leonard Bell	AXJ-114CPCN2RCE	4891

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Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
GAMBEL, PHILLIP	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
08/22/2016	ELECTRONIC

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

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

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

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 13/335,518	Applicant(s) BELL ET AL.	
	Examiner PHILLIP GAMBEL	Art Unit 1644	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 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

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

Status

1)☒ Responsive to communication(s) filed on *03/12/2015*.

☐ 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) *131,133-136,138-141 and 143-146* is/are pending in the application.

5a) Of the above claim(s) *134,135,139,140,144 and 145* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *131,133,136,138,141, 143, 146* 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/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

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

4) ☐ Other: _____.

DETAILED ACTION

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

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office Action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 03/12/2015 has been entered.

Applicant's amendment, filed 03/12/2015, has been entered.

Claims 131, 136 and 141 have been amended.

Claims 152-153 have been added.

Claims 1-130, 132, 137, 142 and 147-153 have been canceled.
Claims 1-130, 132, 137, 142 and 148 have been canceled previously.

Claims 131, 133-136, 138-141 and 143-146 are pending.

As indicated previously, applicant's election of the species (A) (methods of administering anti-C5 antibody, where the claimed do not comprise further administering one or more compounds that increase hematopoiesis in the Response to Restriction Requirement, filed 12/09/2013, has been acknowledged.

Applicant's remarks concerning entitlement of claims to additional species upon allowance of a generic linking claim have been acknowledged.

Claims 131, 133, 136, 138, 141, 143 and 146 are under consideration in the instant application as they read on the elected species.

Claims 134, 135, 139, 140, 144 and 145 have been withdrawn as being drawn to the non-elected species.

3. The text of those sections of Title 35 USC not included in this Office Action can be found in a prior Office Action.

This Office Action will be in response to applicant's arguments and amendments, filed 03/12/2015.

The rejections of record can be found in the previous Office Action, mailed 09/12/2014.

4. Priority.

Applicant's arguments in conjunction with the amended claims, filed 03/12/2015, with respect to priority are acknowledged.

Given the amended claims, filed 03/12/2015, the following is noted.

Newly amended claims 131, 136 and 141 are set forth herein.

131. A method for treating paroxysmal nocturnal hemoglobinuria (PNH) in a patient, the method comprising:
(1) identifying a PNH patient who experiences serum hemolytic activity on a dosing schedule of eculizumab comprising: (i) 600 mg of eculizumab each week for four weeks; (ii) 900 mg of eculizumab on the fifth week; and thereafter, (iii) 900 mg of eculizumab once every two weeks; and
(2) administering 900 mg of eculizumab to the patient once every 12 days to maintain a serum concentration of eculizumab of approximately 35 µg/ml to 50 µg/ml, thereby treating PNH in the patient.

136. A method for treating paroxysmal nocturnal hemoglobinuria (PNH) in a patient, the method comprising:
(1) identifying a PNH patient who experiences serum hemolytic activity on a dosing schedule of eculizumab comprising: (i) 600 mg of eculizumab each week for four weeks; (ii) 900 mg of eculizumab on the fifth week; and thereafter, (iii) 900 mg of eculizumab once every two weeks; and
(2) administering 900 mg of eculizumab to the patient once every 12 days to maintain in the patient a serum complement activity at a level below 20% of the normal serum complement activity, thereby treating PNH in the patient.

141. A method for treating paroxysmal nocturnal hemoglobinuria (PNH) in a patient, the method comprising administering eculizumab to the patient according to the following dosing schedule:
(i) 600 mg of eculizumab each week for four weeks;
(ii) 900 mg of eculizumab on the fifth week; and thereafter,
(iii) 900 mg of eculizumab ~~at least~~ once every 12 days,
wherein the dosing schedule is effective to maintain in the patient a serum complement activity at a level below 20% of the normal serum complement activity, thereby treating PNH in the patient.

Note that <u>not</u> all current claims receive benefit of the previous priority applications.

The recitation of "identifying step" in claims 131 and 136,
"administering once every 12 days to maintain a serum concentration ... / administering once every 12 day to maintain in the patient a serum complement activity …", and
"to maintain a serum concentration of eculizumab of approximately 35 µg/ml to 50 µg/ml"
broadly recited in the newly amended / added claims do <u>not</u> appear to have sufficient written support in the specification as filed.

In addition, the recitation of "wherein the dosing schedule is effective to maintain in the patient a serum complement activity at a level below 20% of the normal serum complement activity, thereby treating PNH in the patient" in claims 141 / 146 is <u>not</u> readily apparent in pages 36-37, overlapping paragraph of the specification (Examples).
The instant application does <u>not</u> particularly identify this particular combination of limitations.

Applicant's remarks, filed 03/12/2015, indicate that the support for the new and amended claims can be found throughout the specification and claims as originally filed.

The written support of the term "identifying" itself is not found in the specification as filed as well as the priority documents.

The written support of the claimed "identifying steps" is not found in the specification as filed as well as the priority documents.

Given the amended claims, it appears that applicant continues to rely upon the Examples to support the current amended claims.

While the term / step of identifying reads on establish or indicating who / someone or what / something is or associated with,

the Example discloses patients who participated in therapy trials to evaluate the effect of anti-C5 antibody on PNH in the absence of clearly disclosing "identifying step(s)" and in the absence of applicant's remarks clearly directing the written support of "identifying" / "identifying steps" in the specification.

Page 36, paragraph 3 of the specification (Examples) discloses the range of "35 µg/ml to *350* µg/ml", not "approximately 35 µg/ml to *50* µg/ml"

in the context of serum hemolytic activity in nine of the elven patients as being completely blocked throughout the 64 week treatment period of trough levels of eculizumab at equilibrium ranging …" rather "to maintain a serum concentration of eculizumab …" as currently claimed.

Pages 36-37, overlapping paragraph of the specification (Examples) disclosing that a reduction of eculizumab above 35 µg//ml and effectively and consistently blocked serum hemolytic activity for the remainder of the extension study for both patients who did not sustain levels of eculizumab necessary to consistently block complement rather than "administering to the patient once every 12 days to maintain a serum complement activity at a level below 20% of the normal serum complement activity …" as currently claimed.

Applicant's reliance on generic disclosure and possibly certain observations in the instant Examples of eleven PNH patients who were transfusion-dependent and hemolytic in therapy trials and who were given a particular therapeutic regimen of eculizumab, including excerpt derived from pages 36-37 of the specification does not provide sufficient written description nor direction and guidance to the "currently claimed limitations".

Note that the explicit recitation of the "limitations" cited above are not readily apparent in the specification as filed.

Note that applicant has amended the claims by relying upon certain observations of the eleven patients either in the treatment period or in an extension study under certain distinct dosing variable and time periods to broadly claim new limitations that departs from the instant disclosure as filed.

The instant disclosure of does not define nor set forth parameters" or provide for the metes and bounds of the "identifying steps".

Obviousness is not the standard for the addition new limitations to the disclosure as filed.
It is noted that entitlement to a filing date does not extend to subject matter which is not disclosed, but would be obvious over what is expressly disclosed. Lockwood v. American Airlines Inc., 41 USPQ2d 1961 (Fed. Cir. 1977).

It is noted that a generic or a sub-generic disclosure cannot support a species unless the species is specifically described. It cannot be said that a subgenus is necessarily described by a genus encompassing it and a species upon which it reads. See In re Smith 173 USPQ 679, 683 (CCPA 1972) and MPEP 2163.05.

The instant claims now recite limitations which were not clearly disclosed in the specification as-filed, and now change the scope of the instant disclosure as-filed. Applicant is claiming a subgenus not supported by the specification as-filed. Such limitations recited in the present claims, which did not appear in the specification, as filed, introduce new concepts and violate the description requirement of the first paragraph of 35 U.S.C. 112.

It is also true that the written description requirement must still be met in some way so as to "describe the claimed invention so that one skilled in the art can recognize what is claimed".

A patent specification should set forth enough detail to allow a person or ordinary skill in the art to understand what is claimed and to recognize that the inventor invented what is claimed.

If applicant desires priority for the above-mentioned limitation / current claims prior to filing date of the instant application (12/22/2011);
applicant is invited to point out and provide documentary support for the priority of the instant claims.

Applicant is reminded that such priority for the instant limitations requires written description and enablement under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph.

5. Claims 131, 133, 136 and 146 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claims 131, 133, 136 and 138 are indefinite in the recitation of "the identifying steps…" because these "limitations" are not clearly defined by the claim and the specification does not provide a sufficient standard for ascertaining the requisite degree or endpoints to reasonably apprise one of ordinary skill in the art of the metes and bounds of the invention.

For example, the specification does not set forth nor disclose the term "identifying" / "identifying steps" and does not define the metes and bounds nor the nature of the particular "identifying steps" encompassed by this claimed recitation.

While applicant's remarks, filed 03/12/2015 in response to the previous rejection under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph relies upon the current amended claims to address the issue of positive steps by specifying the steps of (1) identifying and (2) administering,
applicant does not provide sufficient written description of the identifying steps in the specification (and priority documents) as filed.

It is noted that this "identifying steps" do not recite any additional active method steps, but simply state a characterization or conclusion of steps (not claimed) or may be performed entirely in the human mind is obviously not tied to any machine and do not transform any article into a different state or thing.
For example, the identifying steps read on mental steps which do not require positive steps.

The instant specification does not provide a sufficient definition nor description of the appropriate steps and readouts to apprise the ordinary artisan of the metes and bounds of the claimed "identifying limitations" indicated herein.

It is not clear as what positive steps, measures / parameters, etc., occur other than mental steps, including oral / written communications such as being informed by another or reading a report.

In the absence of a disclosure / description and/or a clear definition in the specification as filed,
the claimed recitation of the "identifying steps" is indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicant is reminded that the amendment must point to a basis in the specification so as not to add any new matter. See MPEP 714.02 and 2163.06.

6. This is a written description rejection, new matter, under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph.

Claims 131, 133, 136, 138, 141, 143 and 146 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 131, 136 and 141 are set forth herein.

131. A method for treating paroxysmal nocturnal hemoglobinuria (PNH) in a patient, the method comprising:
(1) identifying a PNH patient who experiences serum hemolytic activity on a dosing schedule of eculizumab comprising: (i) 600 mg of eculizumab each week for four weeks; (ii) 900 mg of eculizumab on the fifth week; and thereafter, (iii) 900 mg of eculizumab once every two weeks; and
(2) administering 900 mg of eculizumab to the patient once every 12 days to maintain a serum concentration of eculizumab of approximately 35 µg/ml to 50 µg/ml, thereby treating PNH in the patient.

136. A method for treating paroxysmal nocturnal hemoglobinuria (PNH) in a patient, the method comprising:
(1) identifying a PNH patient who experiences serum hemolytic activity on a dosing schedule of eculizumab comprising: (i) 600 mg of eculizumab each week for four weeks; (ii) 900 mg of eculizumab on the fifth week; and thereafter, (iii) 900 mg of eculizumab once every two weeks; and
(2) administering 900 mg of eculizumab to the patient once every 12 days to maintain in the patient a serum complement activity at a level below 20% of the normal serum complement activity, thereby treating PNH in the patient.

141. A method for treating paroxysmal nocturnal hemoglobinuria (PNH) in a patient, the method comprising administering eculizumab to the patient according to the following dosing schedule:
(i) 600 mg of eculizumab each week for four weeks;
(ii) 900 mg of eculizumab on the fifth week; and thereafter,
(iii) 900 mg of eculizumab ~~at least~~ once every 12 days,
wherein the dosing schedule is effective to maintain in the patient a serum complement activity at a level below 20% of the normal serum complement activity, thereby treating PNH in the patient.

The recitation of "identifying step" in claims 131 and 136,
"administering once every 12 days to maintain a serum concentration ... / administering once every 12 day to maintain in the patient a serum complement activity …", and
"to maintain a serum concentration of eculizumab of approximately 35 µg/ml to 50 µg/ml"
broadly recited in the newly amended / added claims do not appear to have sufficient written support in the specification as filed.

In addition, the recitation of "wherein the dosing schedule is effective to maintain in the patient a serum complement activity at a level below 20% of the normal serum complement activity, thereby treating PNH in the patient" in claims 141 / 146 is not readily apparent in pages 36-37, overlapping paragraph of the specification (Examples).
The instant application does not particularly identify this particular combination of limitations.

Applicant's remarks, filed 03/12/2015, indicate that the support for the new and amended claims can be found throughout the specification and claims as originally filed.

The written support of the term "identifying" itself is not found in the specification as filed as well as the priority documents.
The written support of the claimed "identifying steps" is not found in the specification as filed as well as the priority documents.

Given the amended claims, it appears that applicant continues to rely upon the Examples to support the current amended claims.

While the term / step of identifying reads on establish or indicating who / someone or what / something is or associated with,

the Example discloses patients who participated in therapy trials to evaluate the effect of anti-C5 antibody on PNH in the absence of clearly disclosing "identifying step(s)" and in the absence of applicant's remarks clearly directing the written support of "identifying" / "identifying steps" in the specification.

Page 36, paragraph 3 of the specification (Examples) discloses the range of "35 µg/ml to *350* µg/ml, not "approximately 35 µg/ml to *50* µg/ml"

in the context of serum hemolytic activity in nine of the elven patients as being completely blocked throughout the 64 week treatment period of trough levels of eculizumab at equilibrium ranging …" rather "to maintain a serum concentration of eculizumab …" as currently claimed.

Pages 36-37, overlapping paragraph of the specification (Examples) disclosing that a reduction of eculizumab above 35 µg//ml and effectively and consistently blocked serum hemolytic activity for the remainder of the extension study for both patients who did not sustain levels of eculizumab necessary to consistently block complement rather than "administering to the patient once every 12 days to maintain a serum complement activity at a level below 20% of the normal serum complement activity …" as currently claimed.

In addition, the recitation of "wherein the dosing schedule is effective to maintain in the patient a serum complement activity at a level below 20% of the normal serum complement activity, thereby treating PNH in the patient" in claims 141 / 146 is not readily apparent in pages 36-37, overlapping paragraph of the specification (Examples).

The instant application does not particularly identify this particular combination of limitations.

Applicant's reliance on generic disclosure and possibly certain observations in the instant Examples of eleven PNH patients who were transfusion-dependent and hemolytic in therapy trials and who were given a particular therapeutic regimen of eculizumab, including excerpt derived from pages 36-37 of the specification does not provide sufficient written description nor direction and guidance to the "currently claimed limitations".

Note that the explicit recitation of the "limitations" cited above are not readily apparent in the specification as filed.

Note that applicant has amended the claims by relying upon certain observations of the eleven patients either in the treatment period or in an extension study under certain distinct dosing variable and time periods to broadly claim new limitations that departs from the instant disclosure as filed.

The instant disclosure of does not define nor set forth parameters" or provide for the metes and bounds of the "identifying steps".

Obviousness is not the standard for the addition new limitations to the disclosure as filed.

It is noted that entitlement to a filing date does not extend to subject matter which is not disclosed, but would be obvious over what is expressly disclosed. Lockwood v. American Airlines Inc., 41 USPQ2d 1961 (Fed. Cir. 1977).

It is noted that a generic or a sub-generic disclosure cannot support a species unless the species is specifically described. It cannot be said that a subgenus is necessarily described by a genus encompassing it and a species upon which it reads. See In re Smith 173 USPQ 679, 683 (CCPA 1972) and MPEP 2163.05.

The instant claims now recite limitations which were not clearly disclosed in the specification as-filed, and now change the scope of the instant disclosure as-filed. Applicant is claiming a subgenus not supported by the specification as-filed. Such limitations recited in the present claims, which did not appear in the specification, as filed, introduce new concepts and violate the description requirement of the first paragraph of 35 U.S.C. 112.

It is also true that the written description requirement must still be met in some way so as to "describe the claimed invention so that one skilled in the art can recognize what is claimed".

A patent specification should set forth enough detail to allow a person or ordinary skill in the art to understand what is claimed and to recognize that the inventor invented what is claimed.

Applicant is reminded that such priority for the instant limitations requires written description and enablement under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph.

7. Upon reconsideration of applicant's amended claims and arguments, filed 03/12/2015, the previous rejection rejected under 35 U.S.C. § 102(b) as being anticipated by Hillmen et al. (Blood 102: 1858, 2003) has been withdrawn in view of the recitation of an identifying step and an administering 900 mg eculizumab to the patient every 12 days step has been withdrawn.

8. Upon reconsideration of applicant's amended claims and arguments, filed 03/12/2015, the previous rejection rejected under 35 U.S.C. § 102(b) as being anticipated by Hillmen (44th ASH Annual Meeting Abstract, Blood, Vol. 100, 2002) has been withdrawn in view of the recitation of an identifying step and an administering 900 mg eculizumab to the patient every 12 days step.

9. Upon reconsideration of applicant's amended claims and arguments, filed 03/12/2015, the previous rejection under 35 U.S.C. § 103(a) as being unpatentable over Hillmen et al. (Blood 102: 1858, 2003) (1449) AND/OR Hillmen (44th ASH Annual Meeting Abstract, Blood, Vol. 100, 2002) (1449) in view of Meyers et al. (Int. J. Hematol. 77: 125-132, 2003) (1449) and as evidenced by Garba et al. (Singapore Med. J. 46(11):632-634, 2005) (1449) with respect to normal LDH levels has been withdrawn in view of the recitation of an identifying step and/or an administering 900 mg eculizumab to the patient every 12 days step.

10. New Grounds of Rejection

Claims 131, 133, 136, 138, 141, 143 and 146 are rejected under 35 U.S.C. § 102(b) as being anticipated by Bell et al. US 2005/0191298) (1449) (see entire document).

Given the priority issues set forth above,
this rejection is set forth.

Bell et al. teach the methods of treating PNH with eculizumab, including the dosing regimens recited in the instant claims, including the characterization of the patients and serum complement activity in patents (see entire document, including Abstract, Background, Summary, Detailed Description, particularly paragraphs [0065]-[0067], Examples in paragraphs [0081]-[0096],Claims and Figures).

While Bell et al. teach the same methods as claims,
it is acknowledged that Bell et al. differs from the claims by not reciting the "identifying steps" per se.

However, it is noted that this "identifying steps" do not recite any additional active method steps, but simply state a characterization or conclusion of steps (not claimed) or may be performed entirely in the human mind is obviously not tied to any machine and do not transform any article into a different state or thing.
For example under the broadest reasonable interpretation of the claims,
the identifying steps read on mental steps which do not require positive steps.

Again, the referenced Examples teach the same methods as claimed and that the prior art teachings of the administering step would have been those patients identified / chosen / selected by the physicians in the clinical trials taught by the prior art.

It does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure
In turn, the claimed limitations are explicitly or inherent in the prior art teachings.

11. New Ground of Rejection.

Claims 131, 133, 136, 138, 141, 143 and 146 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Bell et al. US 2005/0191298) (1449) (see entire document).

Given the priority issues set forth above,
this rejection is set forth.

Bell et al. teach the methods of treating PNH with eculizumab, including the dosing regimens recited in the instant claims, including the characterization of the methods (see entire document, including Abstract, Background, Summary, Detailed Description, particularly paragraphs [0065]-[0067], Examples in paragraphs [0081]-[0096],Claims and Figures).

While Bell et al. teach the same methods as claims,
it is acknowledged that Bell et al. differs from the claims by not reciting the "identifying steps" per se.

However, it is noted that this "identifying steps" do not recite any additional active method steps, but simply state a characterization or conclusion of steps (not claimed) or may be performed entirely in the human mind is obviously not tied to any machine and do not transform any article into a different state or thing.
For example under the broadest reasonable interpretation of the claims,
the identifying steps read on mental steps which do not require positive steps.

Again, the referenced Examples teach the same methods as claimed and that the prior art teachings of the administering step would have been those patients identified / chosen / selected by the physicians in the clinical trials taught by the prior art.

It does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure
In turn, the claim limitations are explicitly or inherent / intrinsic in the prior art teachings.

Alternatively, one of ordinary skill in the art at the time the invention was made would have been motivated identify those patients who were treated with the regimen in claims 131(1) and 136(1) as taught in the Examples and then administer 900 mg eculizumab once every 12 days also taught by the Examples to maintain eculizumab concentrations and/or to inhibit serum hemolytic activity in the treatment of PNH (e.g., see Examples, including paragraph [0088].

It does not appear that the claim language or limitations result in an unobvious manipulative difference in the method steps when compared to the prior art disclosure of providing 600 mg weekly for four (4) doses and 900 mg every other week of eculizumab a humanized anti-C5 antibody and maintenance doses in the treatment of patients with PNH at the time the invention was made.

Given the nature of the clinical manifestations of PNH such as hemolysis and anemia, one of ordinary skill in the art at the time the invention was made would have been motivated to identify / select / determine those patients who did not sustain levels of eculizmab necessary to consistenly block complement by administering 900 mg eculizumab once every 12 days also taught by the Examples to maintain eculizumab concentrations and/or to inhibit serum hemolytic activity in the treatment of PNH (e.g., see Examples, including paragraph [0088 select and combine both anti-C5 antibodies and erythropoietin to address and manage the clinical manifestations of PNH over time with an expectation of success.

From the teachings of the references, it was apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

"The test of obviousness is not express suggestion of the claimed invention in any or all of the references but rather what the references taken collectively would suggest to those of ordinary skill in the art presumed to be familiar with them." See In re Rosselet, 146 USPQ 183, 186 (CCPA 1965).

"There is no requirement (under 35 USC 103(a)) that the prior art contain an express suggestion to combine known elements to achieve the claimed invention. Rather, the suggestion to combine may come from the prior art, as filtered through the knowledge of one skilled in the art." Motorola, Inc. v. Interdigital Tech. Corp., 43 USPQ2d 1481, 1489 (Fed. Cir. 1997).

An obviousness determination is not the result of a rigid formula disassociated from the consideration of the facts of a case. Indeed, the common sense of those skilled in the art demonstrates why some combinations would have been obvious where others would not. See KSR Int'l Co. v. Teleflex Inc., 82 USPQ2d 1385 (U.S. 2007).

12. No claim is allowed.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (571) 272-0844. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached on (571) 272-3181.

The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Phillip Gambel/
Primary Examiner
Technology Center 1600
Art Unit 1644
August 17, 2016

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/335,518	12/22/2011	Leonard Bell	AXJ-114CPCN2	4891

959 7590 09/12/2014

NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

EXAMINER	
GAMBEL, PHILLIP	
ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
09/12/2014	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):

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 13/335,518	Applicant(s) BELL ET AL.	
	Examiner PHILLIP GAMBEL	Art Unit 1644	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 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

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

Status

1)☒ Responsive to communication(s) filed on *07/07/2014*.

☐ 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) *131,133-136,138-141,143-147 and 149-153* is/are pending in the application.

5a) Of the above claim(s) *134, 135, 139, 140, 144, 145, 150 and 151* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *131,133,136,138,141,143,146,147,149,152 and 153* 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/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

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

4) ☐ Other: _____.

DETAILED ACTION

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

2. Applicant's amendment, filed 07/07/2014, has been entered.

Claims 131, 136, 141 and 147 have been amended.

Claims 152-153 have been added.

Claims 132, 137, 142 and 148 have been canceled.
Claims 1-130 have been canceled previously.

Claims 131, 133-136, 138-141, 143-147 and 149-153 are pending.

As indicated previously, applicant's election of the species (A) (methods of administering anti-C5 antibody, where the claimed do not comprise further administering one or more compounds that increase hematopoiesis in the Response to Restriction Requirement, filed 12/09/2013, has been acknowledged.

Applicant's remarks concerning entitlement of claims to additional species upon allowance of a generic linking claim have been acknowledged.

Claims 131, 133, 136, 138, 141, 143, 146, 147, 149, 152 and 153 are under consideration in the instant application as they read on the elected species.

Claims 134, 135, 139, 140, 144, 145, 150 and 151 have been withdrawn as being drawn to the non-elected species.

3. The text of those sections of Title 35 USC not included in this Office Action can be found in a prior Office Action.

This Office Action will be in response to applicant's arguments and amendments, filed 07/07/2014).

The rejections of record can be found in the previous Office Action, mailed 02/06/2014.

4. Priority.

Note that not all claims may receive benefit of the priority applications.

The recitation of "the method comprising administering eculizumab to a patient who has experienced complement-related serum hemolytic activity breakthrough ...",
"a method for preventing complement-related serum hemolytic activity breakthrough in a patient",
"with a frequency effective to maintain a serum concentration of eculizumab of at least 35 µg/ml",
"at least once every 12 days to maintain in the patient a serum complement activity at a level below 20% of the normal serum complement activity"
broadly recited in the newly amended / added claims do not appear to have sufficient written support in the specification as filed.

Applicant's remarks, filed 07/07/2014, indicate that the support for the new and amended claims can be found throughout the specification and claims as originally filed.

Applicant also relies upon the following with respect to Priority.

Applicants respectfully note that literal support for an administration regimen that comprises administration of an anti-C5 antibody at least once every 12 days can be found at least, for example, at page 36 (line 16) through page 37 (line 9) of the specification as originally filed. For the Examiner's convenience, the relevant section is reproduced below:

Serum hemolytic activity in nine of the eleven patients was completely blocked throughout the 64 week treatment period with trough levels of eculizumab at equilibrium ranging from approximately 35 µg/ml to 350 µg/ml. During the extension study, 2 patients did not sustain levels of eculizumab necessary to consistently block complement. This breakthrough in serum hemolytic activity occurred in the last 2 days of the 14 day dosing interval, a pattern that was repeated between multiple doses. In one of the patients, as seen in FIG. 4, break-through of complement blockade resulted in hemoglobinuria, dysphagia, and increased LDH and AST, which correlated with the return of serum hemolytic activity. At the next dose, symptoms resolved (FIG. 5) and reduction in the dosing interval from 900 mg every 14 days to 900 mg every 12 days resulted in a regain of complement control which was maintained over the extension study to 64 weeks in both patients. This patient showed a 24 hour resolution of dysphagia and hemoglobinuria. A reduction in the dosing interval from 14 to 12 days was sufficient to maintain levels of eculizumab above 35 µg/ml and effectively and consistently blocked serum hemolytic activity for the remainder of the extension study for both patients (emphasis added).

Applicants also confirm that the present application is not a continuation-in- part of the prior application (USSN 12/902,617), but rather a continuation of USSN 12/902,617, which is a continuation of USSN 11/050,543, which is a continuation-in-part of USSN 10/771,552, as specified on the Application Data Sheet as filed with the application. Accordingly, the same support for "at least once every 12 days" that exists in the present specification can be found in each of the priority applications, including the earliest priority application, USSN 10/771,552 (see, e.g., paragraph [0078] of the corresponding published application, U.S. 2005/0169921).

Applicants, therefore, respectfully maintain that the presently pending claims are entitled to full priority.

Applicant's reliance on generic disclosure and possibly certain observations in the instant Example of eleven PNH patients who were transfusion-dependent and hemolytic in therapy trials and who were given a particular therapeutic regimen of eculizumab, including excerpt derived from pages 36-37 of the specification does not provide sufficient written description nor direction and guidance to the "currently claimed limitations".

Note that the explicit recitation of the "limitations" cited above are not readily apparent in the specification as filed.

Note that applicant has amended the claims or added new claims by relying upon certain observations of the eleven patients either in the treatment period or in an extension study under certain distinct dosing variable and time periods to broadly claim new limitations.

Again, the recitation of "comprising administering to a patient with PNH an anti-C5 antibody at least once every 12 days to thereby maintain in the patient a serum complement activation at a level below 20% of the normal serum complement activity", "at least once every 12 days" and "at least 35 μg/mg/ml" are setting forth new limitations based upon certain observations of the Examples, including setting forth limitations with no upper limit that departs from the instant disclosure as filed.

The limited disclosure of "a breakthrough in serum hemolytic activity" does not define nor set forth parameters for the "breakthrough" or provide for the metes and bounds of said "breakthrough".

The specification as filed does not provide sufficient written description of a separate category of patients who has experience complement-related serum hemolytic breakthrough, nor prevent PNH in such patients based upon certain observations in the clinical trials of the Example.

Obviousness is not the standard for the addition new limitations to the disclosure as filed. It is noted that entitlement to a filing date does not extend to subject matter which is not disclosed, but would be obvious over what is expressly disclosed. Lockwood v. American Airlines Inc., 41 USPQ2d 1961 (Fed. Cir. 1977).

It is noted that a generic or a sub-generic disclosure cannot support a species unless the species is specifically described. It cannot be said that a subgenus is necessarily described by a genus encompassing it and a species upon which it reads. See In re Smith 173 USPQ 679, 683 (CCPA 1972) and MPEP 2163.05.

The instant claims now recite limitations which were not clearly disclosed in the specification as-filed, and now change the scope of the instant disclosure as-filed. Applicant is claiming a subgenus not supported by the specification as-filed. Such limitations recited in the present claims, which did not appear in the specification, as filed, introduce new concepts and violate the description requirement of the first paragraph of 35 U.S.C. 112.

It is also true that the written description requirement must still be met in some way so as to "describe the claimed invention so that one skilled in the art can recognize what is claimed.

A patent specification should set forth enough detail to allow a person or ordinary skill in the art to understand what is claimed and to recognize that the inventor invented what is claimed.

If applicant desires priority for the above-mentioned limitation / current claims prior to filing date of the instant application (12/22/2011);
applicant is invited to point out and provide documentary support for the priority of the instant claims.

Applicant is reminded that such priority for the instant limitations requires written description and enablement under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph.

Therefore, this application repeats a substantial portion of prior USSN 12/902,617, filed 10/12/2010 and adds and claims additional disclosure not presented in the prior application, as indicated above.

Since this application names an inventor or inventors named in the prior application, it may constitute a continuation-in-part of the prior application.

Should applicant desire to obtain the benefit of the filing date of the prior application, attention is directed to 35 U.S.C. 120 and 37 CFR 1.78.

Therefore, applicant should amend the first line of the specification to indicate the status of the instant application as a continuation-in-part and file an Application Data Sheet reflecting the proper relationship of the instant application to its priority documents.

A claim as a whole has only one effective filing date.
See Studiengelsellschaft Kahle m.b.H. v. Shell Oil Co. 42 USPQ2d 1674, 1677 (Fed. Cir 1997).

Applicant's arguments, filed 07/14/2014, have been fully considered but have not been found convincing essentially for the reasons of record.

5. Upon reconsideration of applicant's amended claims, filed 07/07/2014, the previous rejection under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, with respect to the "wherein clauses" have been withdrawn.

6. Claims 131, 133, 136, 138, 141, 143, 146, 149, 152 and 153 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claims 131-133, 136-138, 141-143 and 146-149 are indefinite in the recitation of "a patient who has experienced complement-related serum hemolytic activity breakthrough" to maintain a serum concentration of eculizumab" because it is not clear as to what are the parameters that define specific patient populations (e.g., to be treated or have already been treated) or defines other characteristics of the claimed methods of elements recited within the claimed methods (e.g., amounts and frequency) of the claimed methods.

Applicant's reliance on generic disclosure and possibly certain observations in the instant Example of eleven PNH patients who were transfusion-dependent and hemolytic in therapy trials and who were given a particular therapeutic regimen of eculizumab, including excerpt derived from pages 36-37 of the specification does not 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 to the "currently claimed limitations", as argued / asserted by applicant.

Other than relying upon the observations of the clinical and extend trails on pages 56-57 of the specification,
the specification does not define nor set forth the parameters of "a patient who has experienced complement-related serum hemolytic activity breakthrough".

Given the nature of the patients populations being treated (e.g., those with PNH or having symptoms associated with PNH) as well as applicant's reliance upon the Example for this limitation, wherein the Example describes certain patients undergoing treatment in clinical trials and extended trials,
it is unclear or indefinite as to the metes and bound of the claimed patient populations to be treated, that is, the broadest reasonable interpretation of the claims read on treating PNH patients, PNH patients undergoing treatment, who are PNH patients generally and/or patients with symptoms of PNH,
rather than being limited to patients identified as having experienced complement-related serum hemolytic activity breakthrough, as argued / asserted by applicant.

There is no positive step with regard to "identifying".

There is no clear definition, nor parameters as well as explicit recitation of "a patient who has experienced complement-related serum hemolytic activity breakthrough" or even "complement-related serum hemolytic activity breakthrough" in the specification as filed.

It is unclear whether the claimed methods necessarily limits the claimed patient population to a very well defined patient populations or simply states a characterization or conclusion of the results of those steps of patient populations being treated for PNH or having symptoms / manifestations such as hemolytic anemia associated with PNH with anti-C5 antibodies / eculizumab.

The claims are 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.

Applicant is reminded that the amendment must point to a basis in the specification so as not to add any new matter. See MPEP 714.02 and 2163.06

7. The specification is objected to as failing to provide proper antecedent basis for the claimed subject matter. See 37 C.F.R. § 1.75(d)(1) and M.P.E.P. § 608.01(o). Correction of the following is required:

The recitation of "comprising administering to a patient with PNH an anti-C5 antibody at least once every 12 days to thereby maintain in the patient a serum complement activation at a level below 20% of the normal serum complement activity" and "at least once every 12 days" (e.g., see claims 136 and dependent claims thereof, claims 141 and dependent claims thereof).

Applicant's arguments, filed 07/14/2014, have been fully considered but have not been found convincing essentially for the reasons of record.

In contrast to applicant's reliance upon pages 36-37 of the instant specification based upon the specifics of eleven patients participating in therapy and extended trials evaluating the effects of anti-C5 antibody / eculizumab on PNH and symptoms associated therewith,
the claims associated with the preliminary amendment, filed 12/22/2011, including the recitation of "at least once every 12 days" is not readily apparent in the specification as-filed and broadens the instant disclosure as-filed.

Applicant's preliminary amendment, filed 12/22/2011, directs support for the newly added claims to various pages of the specification but does not provide clear direct support for the above-mentioned limitations encompassing administration regimens that includes the recitation of "at least once every 12 days".

The written support of administration regimen that includes the recitation of "at least once every 12 days" is not readily apparent in the specification as-filed.

Applicant's arguments have not been found persuasive.

Applicant is required to amend the specification to provide proper antecedent basis of the current claims in the specification.

Alternatively, applicant is invited to identify the written support for instant claims in the specification as filed.

8. This is a written description rejection, new matter under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph.

Claims 131,133, 136, 138, 141, 143, 146, 147, 152 and 153 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicant's arguments, filed 07/14/2014, have been fully considered but have not been found convincing essentially for the reasons of record.

In contrast to applicant's reliance upon pages 36-37 of the instant specification based upon the specifics of eleven patients participating in therapy and extended trials evaluating the effects of anti-C5 antibody / eculizumab on PNH and symptoms associated therewith,
applicant's reliance on generic disclosure and possibly certain observations in the instant Example of eleven PNH patients who were transfusion-dependent and hemolytic in therapy trials and who were given a particular therapeutic regimen of eculizumab, including excerpt derived from pages 36-37 of the specification does not provide sufficient written description nor direction and guidance to the "currently claimed limitations".

Applicant relies upon the observations of treating a particular patient undergoing a particular dosing regimen on pages 36-37 of the specification to establish a new imitation.

A reduction in the dosing interval from 14 to 12 days was sufficient to maintain levels of eculizumab above 35 µg/ml and effectively and consistently blocked serum hemolytic activity for the remainder of the extension study for both patients.

Note that the explicit recitation of the "limitations" cited above are not readily apparent in the specification as filed.

Note that applicant has amended the claims or added new claims by relying upon certain observations of the eleven patients either in the treatment period or in an extension study under certain distinct dosing variable and time periods to broadly claim new limitations.

The specification as originally filed does not provide support for the invention as now claimed:

"at least 35 μg/ml" and "wherein the patient has experienced complement-related serum hemolytic activity breakthrough".

As indicated previously and consistent with current arguments,

applicant's amendment, filed 12/09/2013, directs support for the newly amended claims to paragraphs [0012], [0081], [0088] and [0093] of the corresponding US2012/0308559 and asserts no new matter has been filed.

Again, as noted previously, the specification as-filed appears to provide written description of "approximately 35 μg/ml to 50 μg/ml" and not an open-ended range of "at least 35 μg/ml" as currently recited (see pages 6-7, overlapping paragraph of the specification' paragraph [0088] of US2012/0308559).

Also, the specification as-filed appears to provide written description of "serum hemolytic activity" and not "complement-related serum hemolytic activity" as currently recited (see pages 6-7, overlapping paragraph of the specification' paragraph [0088] of US2012/0308559).

The newly amended "limitations" have broaden the written description of the Examples relied upon for the claimed "limitations" and, in turn, represent a departure of the specification / application as-filed.

The specification does not provide sufficient blazemarks nor direction for the instant methods encompassing the above-mentioned "limitations" as currently recited. The instant claims now recite limitations which were not clearly disclosed in the specification as-filed, and now change the scope of the instant disclosure as-filed. Such limitations recited in the present claims, which did not appear in the specification, as filed, introduce new concepts and violate the description requirement of the first paragraph of 35 U.S.C. 112.

Applicant is required to cancel the new matter in the response to this Office Action.

Alternatively, applicant is invited to provide sufficient written support for the "limitations" indicated above.

See MPEP 714.02 and 2163.06.

Applicant's arguments have not been found persuasive.

9. Claims 131, 133, 136, 138, 146, 147, 149, 152 and 153 are rejected under 35 U.S.C. § 102(a)(b) as being anticipated by Hillmen et al. (Blood 102: 1858, 2003) (1449) (see entire document).

Applicant's arguments in conjunction with certain legal citations, filed 07/14/2014, have been fully considered but have not been found convincing essentially for the reasons of record.

In contrast to applicant's arguments that the prior art fails to identify a patient who has experience a complement related serum hemolytic activity breakthrough on a particular dosing regimen schedule, the following is noted.

Under the broadest reasonable interpretation of the claims,
the claims read on treating PNH in patient populations who are patients in need of being treated such as those patients who have a complement-related serum hemolytic activity, who are PNH patients who are undergoing treatment (e.g., treatment with anti-C5 antibody / eculizumab) and/or patients who have been undergoing treatment and who are determined to have complement-related serum hemolytic activity.

Note that the claims do not recite a specific positive step of identifying a patient who has experienced complement-related serum hemolytic activity breakthrough.

Note that PNH is characterized by hemolytic anemia (a decreased number of red blood cells) (e.g., see page 2, paragraph 2 of the specification) and serum hemolytic activity (i.e., the percentage of cell lysis) (e.g., see page 40, paragraph 1 of the specification).

The specification does not define, nor set forth the parameters of "complement-related serum hemolytic activity breakthrough".

Given the description of "breakthrough" on pages 36-37, overlapping paragraphs of the specification,
a breakthrough could read on serum hemolytic activity or serum hemolytic activity that is not consistently block by treatment and/or treatment by eculizumab.

The following is reiterated for applicant's convenience.

Since the public availability of this document is not clear and given that certain claims are subject to a rejection under 35 USC 112, first paragraph, written description / new matter;
this rejection is being applied under 35 U.S.C. § 102(a)(b).

Hillmen et al. teach methods of treating paroxysmal nocturnal hemoglobulinuria (PNH), intravascular hemolysis and venous thrombosis, with 900 mg eculizumab by i.v. infusion every other week for one year an anti-C5 antibody and completing 11 and 16 months of therapy and in certain instances with erythropoietin as well, including improvement in biochemical / biological parameters associated with LDH, PNH type III cells and complement activity (see entire document).

The prior art dosage administration regimen reads on the claimed methods.

Consistent with the rejection under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, above and with respect to claim construction for prior art rejections, the following is noted.

Although applicant has amended the claims to avoid the previously recited "wherein clauses", the claims do not necessarily recite any additional active method steps, but simply states a characterization or conclusion of the results of those steps or the patient populations that have been treated of or had symptoms / manifestations such as hemolytic anemia of PNH.

Therefore, under the broadest reasonable interpretation of the claims, the recitation a patient who has experienced complement-related serum hemolytic activity breakthrough" is not considered to further limit the method defined by the claim and has been given its proper weight in construing the claims of administering to a patient with PNH and/or manifestations of hemolytic anemia associated with PNH an anti-C5 antibody in an amount and with a frequency effective to maintain a serum concentration of at least 35 μg/ml.

It does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure

Although the reference is silent about certain specific claimed limitations per se of a patient having paroxysmal nocturnal hemoglobulnuria (PNH) or effective amounts to maintain serum complement activity at a certain range, it does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure. See Bristol-Myers Squibb Company v. Ben Venue Laboratories 58 USPQ2d 1508 (CAFC 2001). "{i}t is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable". In re Woodruff, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990). The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145.

Given the prior art teaching of treating with 900 mg of eculizumab every other week, the prior art teaches effective amounts of treatment with the anti-C5 antibody eculizumab occurs in a dosing regimen at least once every 12 days.

On this record, it is reasonable to conclude that the same PNH patients are being administered the same active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., LDH, complement activity and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,
it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to administer anti-C5 antibodies to treat PNH patients.

Applicant's arguments have not been found persuasive.

10. Claims 131, 133, 136, 138, 146, 147, 149, 152 and 153 are rejected under 35 U.S.C. § 102(b) as being anticipated by Hillmen (44th ASH Annual Meeting Abstract, Blood, Vol. 100, 2002) (1449) (see entire document).

Applicant's arguments in conjunction with certain legal citations, filed 07/14/2014, have been fully considered but have not been found convincing essentially for the reasons of record and that addressed above and reiterated herein.

In contrast to applicant's arguments that the prior art fails to identify a patient who has experience a complement related serum hemolytic activity breakthrough on a particular dosing regimen schedule, the following is noted.

Under the broadest reasonable interpretation of the claims,
the claims read on treating PNH in patient populations who are patients in need of being treated such as those patients who have a complement-related serum hemolytic activity, who are PNH patients who are undergoing treatment (e.g., treatment with anti-C5 antibody / eculizumab) and/or patients who have been undergoing treatment and who are determined to have complement-related serum hemolytic activity.

Note that the claims do not recite a specific positive step of identifying a patient who has experienced complement-related serum hemolytic activity breakthrough.

Note that PNH is characterized by hemolytic anemia (a decreased number of red blood cells) (e.g., see page 2, paragraph 2 of the specification) and serum hemolytic activity (i.e., the percentage of cell lysis) (e.g., see page 40, paragraph 1 of the specification).

The specification does not define, nor set forth the parameters of "complement-related serum hemolytic activity breakthrough".

Given the description of "breakthrough" on pages 36-37, overlapping paragraphs of the specification,
a breakthrough could read on serum hemolytic activity or serum hemolytic activity that is not consistently block by treatment and/or treatment by eculizumab.

The following of record is reiterated for applicant's convenience.

Hillmen (2002) teaches that paroxysmal nocturnal hemoglubinuria (PNH) is characterized by intravascular hemolysis and venous thrombosis. Hillmen teaches that the hemolysis is complement-mediated and due to the deficiency of complement regulatory proteins on red cells, particularly the terminal complement inhibitor CD59 (see entire document). Hillmen teaches treating transfusion-dependent PNH patients with 600 mg weekly for four (4) doses and 900 mg every other week of ECULIZUMAB, a humanized anti-C5 antibody (see entire publication). Hillmen teaches that the biological parameters of hemolysis dramatically improved or normalized for all patients within a week of starting therapy. Hillmen further teaches that mean LDH decreased greater than 50%, from 4494 +/- 3380 units/liter to 636 +/- 166 units/liter.
The prior art dosage administration regimen reads on the claimed methods

Consistent with the rejection under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, above and with respect to claim construction for prior art rejections,
the following is noted.

Although applicant has amended the claims to avoid the previously recited "wherein clauses",
the claims do not necessarily recite any additional active method steps, but simply states a characterization or conclusion of the results of those steps or the patient populations that have been treated of or had symptoms / manifestations such as hemolytic anemia of PNH.

Therefore, under the broadest reasonable interpretation of the claims,
the recitation a patient who has experienced complement-related serum hemolytic activity breakthrough" is not considered to further limit the method defined by the claim and has been given its proper weight in construing the claims of administering to a patient with PNH and/or manifestations of hemolytic anemia associated with PNH an anti-C5 antibody in an amount and with a frequency effective to maintain a serum concentration of at least 35 μg/ml.

It does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure

Although the reference is silent about certain specific claimed limitations per se of a patient having paroxysmal nocturnal hemoglobulnuria (PNH) or effective amounts to maintain serum complement activity at a certain range,
it does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure. See Bristol-Myers Squibb Company v. Ben Venue Laboratories 58 USPQ2d 1508 (CAFC 2001). "{i}t is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable". In re Woodruff, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990). The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145.

Given the prior art teaching of treating with 900 mg of eculizumab every other week,
the prior art teaches effective amounts of treatment with the anti-C5 antibody eculizumab occurs in a dosing regimen at least once every 12 days.

On this record, it is reasonable to conclude that the same PNH patients are being administered the same active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., LDH, complement activity and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,
it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to administer anti-C5 antibodies to treat PNH patients.

Applicant's arguments have not been found persuasive.

11. Claims 131, 133, 136, 138, 141, 143, 146, 147, 149, 152 and 153 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hillmen et al. (Blood 102: 1858, 2003) (1449) AND/OR Hillmen (44th ASH Annual Meeting Abstract, Blood, Vol. 100, 2002) (1449) in view of Meyers et al. (Int. J. Hematol. 77: 125-132, 2003) (1449) and as evidenced by Garba et al. (Singapore Med. J. 46(11):632-634, 2005) (1449) with respect to normal LDH levels.

Applicant's arguments, filed 07/14/2014, have been fully considered but have not been found convincing essentially for the reasons of record.

Applicant argues the following.

The pending claims are based, at least in part, on the surprising discovery that for a subset of patients afflicted with PNH, the original, established dosing regimen for eculizumab was insufficient to maintain the blockade of systemic complement activity in these patients. As described in the specification, this "[b]reakthrough of complement blockade resulted in hemoglobinuria, dysphagia, and increased LDH and AST, which correlated with a return of serum hemolytic activity" (page 36 (line 22) through page 37 (line 2)). These breakthrough patients were treated with a new dosing schedule - 900 mg of eculizumab every 12 days. Applicants further appreciated that, in order to more effectively treat PNH patients, eculizumab should be administered to the patients in an amount and with a frequency effective to maintain a serum concentration of eculizumab of at least 35 ug/ml (claim 131) or to maintain the hemolytic activity potential of the patient' s serum at a level below 20% of the hemolytic activity of their serum prior to treatment with eculizumab (claims 136, 141, and 147). The serum hemolytic activity was measured using the total human serum complement hemolytic assay described in the working examples. As

described in the specification, patients whose serum hemolytic activity (as measured using the assay) rose above 20% of the pretreatment serum hemolytic activity experienced breakthrough. In other words, Applicants determined a therapeutic threshold for more effective treatment of PNH using eculizumab.

Applicants also discovered that administration of eculizumab every 12 days, as presently claimed, is the optimum regimen for these particular patients. Specifically, as shown in Figure 4, at day 0, i.e., before administration of eculizumab, pharmacodynamic (PD) levels were consistently high, indicating high levels of complement activity in the patient's serum, which in turn correlates with high levels of hemolysis. PD levels again were measured on days 9, 12 and 13 after administration of eculizumab. The measured PD levels consistently show that no hemolysis was found until day 12. However, on day 13, PD levels surprisingly increased dramatically, thereby indicating an abrupt relapse of hemolytic activity in the PNH patients. In particular, the PD levels as shown in the below excerpt from Figure 4 demonstrate the dramatic increase of serum hemolytic activity from day 12 to day 13:

	Day 12	Day 13	Day 14 = day 0
PD	2	56	56

Accordingly, the optimal time window for administration of eculizumab is very narrow and a 12 day dosing interval, as recited in claims 136 and 141, is ideal.

None of the cited references teaches or suggests any particular therapeutic threshold for eculizumab, let alone a serum concentration of eculizumab of at least 35 µg/ml (claim 131) or a level which maintains in the patient a serum complement activity below 20% of the normal serum complement activity (claims 136, 141, and 147). Nor do the cited references teach or suggest any optimal window for administering eculizumab, let alone every 12 days (claims 136 and 141). Therefore, contrary to the Examiner's assertion, one of ordinary skill in the art would not have been motivated to have selected the particularly claimed treatment regimens based on the cited references. Specifically, Hillmen 2002, does not teach PNH breakthrough patients. While Hillmen 2003, mentions breakthrough patients, it does not teach the particularly claimed population of patients, as presently claimed (i. e., who experienced complement-related serum hemolytic activity breakthrough on the particular dosing schedule of eculizumab). Neither Hillmen 2002 nor Hillmen 2003 teach or suggest the 20% serum hemolytic activity threshold, the threshold serum concentration of eculizumab of at least 35 ug/ml, or the particular 12 day dosing schedule, as presently claimed.

Myers et al. and Garba et al., fail to cure the deficiencies of Hillmen 2002 and Hillmen 2002. Specifically neither of these references even discusses breakthrough of PNH patients, let alone a particular serum hemolytic activity threshold (e.g., 20%) or a threshold serum concentration of eculizumab (e.g., at least 35 ug/ml) or the optimal 12 day dosing schedule, as presently claimed.

In sum, none of the cited references would have provided motivation to have selected the presently claimed dosage regimens for preventing PNH breakthrough. Accordingly, the presently claimed methods are patentable over the cited references and Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Also, note the rebuttal above in terms of the broadest reasonable interpretation of the claims as follows.

In contrast to applicant's arguments that the prior art fails to identify a patient who has experience a complement related serum hemolytic activity breakthrough on a particular dosing regimen schedule, the following is noted.

Under the broadest reasonable interpretation of the claims,
the claims read on treating PNH in patient populations who are patients in need of being treated such as those patients who have a complement-related serum hemolytic activity, who are PNH patients who are undergoing treatment (e.g., treatment with anti-C5 antibody / eculizumab) and/or patients who have been undergoing treatment and who are determined to have complement-related serum hemolytic activity.

Note that the claims do not recite a specific positive step of identifying a patient who has experienced complement-related serum hemolytic activity breakthrough.

Note that PNH is characterized by hemolytic anemia (a decreased number of red blood cells) (e.g., see page 2, paragraph 2 of the specification) and serum hemolytic activity (i.e., the percentage of cell lysis) (e.g., see page 40, paragraph 1 of the specification).

The specification does not define, nor set forth the parameters of "complement-related serum hemolytic activity breakthrough".

Given the description of "breakthrough" on pages 36-37, overlapping paragraphs of the specification,
a breakthrough could read on serum hemolytic activity or serum hemolytic activity that is not consistently block by treatment and/or treatment by eculizumab.

Also, note that the prior art teach the same or nearly the same dosages and regimen consistent in the same or nearly the same clinical trials to treat PNH.

While applicant relies upon the instant Example, particularly pages 56-57 of the specification to assert unexpected results,
note that serum hemolytic activity of nine of the eleven patients was completely blocked by eculizumab at equilibrium ranging from approximately 35 µg/ml to 350 µg/ml and
that modifying the dosing interval was sufficient to active equilibrium and blocking hemolytic activity of administering eculizumab,
consistent with the result-effective variables of the prior art rejection relying upon treating the same or nearly the same patients with the same or nearly the same dosing /frequency at the time the invention was made.

The following is reiterated for applicant's convenience.

Hillmen et al. (2003) teach methods of treating paroxysmal nocturnal hemoglobulinuria (PNH), intravascular hemolysis and venous thrombosis, with 900 mg eculizumab by i.v. infusion every other week for one year an anti-C5 antibody and completing 11 and 16 months of therapy and in certain instances with erythropoietin as well, including improvement in biochemical / biological parameters associated with LDH, PNH type III cells and complement activity (see entire document).

Hillmen (2002) teaches that paroxysmal nocturnal hemoglubinuria (PNH) is characterized by intravascular hemolysis and venous thrombosis. Hillmen teaches that the hemolysis is complement-mediated and due to the deficiency of complement regulatory proteins on red cells, particularly the terminal complement inhibitor CD59 (see entire document). Hillmen teaches treating transfusion-dependent PNH patients with 600 mg weekly for four (4) doses and 900 mg every other week of ECULIZUMAB, a humanized anti-C5 antibody (see entire publication). Hillmen teaches that the biological parameters of hemolysis dramatically improved or normalized for all patients within a week of starting therapy. Hillmen further teaches that mean LDH decreased greater than 50%, from 4494 +/- 3380 units/liter to 636 +/- 166 units/liter.

It does not appear that the claim language or limitations result in an unobvious manipulative difference in the method steps when compared to the prior art disclosure of providing 600 mg weekly for four (4) doses and 900 mg every other week of eculizumab a humanized anti-C5 antibody and maintenance doses in the treatment of patients with PNH at the time the invention was made.

Given the prior art teachings of treating with 900 mg of eculizumab every other week, the prior art teaches effective amounts of treatment with the anti-C5 antibody eculizumab occurs in a dosing regimen at least once every 12 days to achieve effective treatment or blocking of hemolytic activity in PNH patients at the time the invention was made.

On this record, it is reasonable to conclude that the same PNH patients are being administered the same active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., LDH, complement activity and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,
it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Further, the mean level of LDH in the PNH patients was more than 1.5 times the upper limit of a normal LDH level in a person, as evidenced by Garba, who discloses that normal LDH in a person is 247.10 +/- 19.0 units/liter (Abstract in particular). The prior art teaching clearly anticipates the claimed invention.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,
it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Meyers et al. teach management issues in the treatment of paroxysmal nocturnal hemoglobinuria (see entire document), including the hallmarks of hemolysis and anemia (e.g., see pages 125-128) as well as the treatment of this syndrome with complement inhibitors such as anti-C5 antibodies particularly for the hemolysis of PNH (see Section 2.5, Complement Inhibitors) and erythropoietin (see Section 2.6, Management of Nonhemolytic Anemia).

In addition, as to the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations",
treating PNH patients characterized by the criteria of the proportion of type III red blood cells, platelet counts, type III granulocyte clone and serum complement activity are result effective variables of controlling hemolysis and reducing the risk of venous thrombosis with anti-C5 antibodies in the treatment of PNH patient populations who are characterized by intravascular hemolyisis, venous thrombosis and aplastic anemia.

It is well settled that "discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art." In re Boesch, 617 F.2d 272, 276, 205 USPQ 215, 219 (CCPA 1980). See also Merck & Co. v. Biocraft Labs. Inc., 874 F.2d 804, 809, 10 USPQ2d 1843, 1847-48 (Fed. Cir. 1989) (determination of suitable dosage amounts in diuretic compositions considered a matter of routine experimentation and therefore obvious).

As the nature of the dosing and therapeutic endpoints were known to the ordinary artisan, it would have been obvious to optimize the frequency of anti-C5 antibodies in order to control hemolysis and the risk of venous thrombosis in a variety of PNH patients.

Given the nature of the clinical manifestations of PNH such as hemolysis and anemia, one of ordinary skill in the art at the time the invention was made would have been motivated to select and combine both anti-C5 antibodies and erythropoietin to address and manage the clinical manifestations of PNH over time. One would have been motivated to combine these therapies with a reasonable expectation of success because each treats a different aspect of the hemolytic disease in the patient and could be reasonably expected to improve the prognosis of the patient more rapidly than either therapy alone. From the teachings of the references, it was apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

"The test of obviousness is not express suggestion of the claimed invention in any or all of the references but rather what the references taken collectively would suggest to those of ordinary skill in the art presumed to be familiar with them." See In re Rosselet, 146 USPQ 183, 186 (CCPA 1965).

"There is no requirement (under 35 USC 103(a)) that the prior art contain an express suggestion to combine known elements to achieve the claimed invention. Rather, the suggestion to combine may come from the prior art, as filtered through the knowledge of one skilled in the art." Motorola, Inc. v. Interdigital Tech. Corp., 43 USPQ2d 1481, 1489 (Fed. Cir. 1997).

An obviousness determination is not the result of a rigid formula disassociated from the consideration of the facts of a case. Indeed, the common sense of those skilled in the art demonstrates why some combinations would have been obvious where others would not. See KSR Int'l Co. v. Teleflex Inc., 82 USPQ2d 1385 (U.S. 2007) ("The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.").

Applicant's arguments have not been found persuasive.

11. Given copending applications encompassing similar subject matter and the restriction set forth herein, applicant is invited to clarify which applications should be subject to rejections under the judicially created doctrine of obviousness-type double patenting.

Applicant's submission of an Information Disclosure Statement to make of record related appliations as wella s Office Actions is acknowledged.

12. No claim is allowed.

13. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, THIS ACTION IS MADE FINAL. See MPEP § 706.07(a). Applicant is 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 date of this final action.

14. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (571) 272-0844. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached on (571) 272-3181.

The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Phillip Gambel/
Primary Examiner
Technology Center 1600
Art Unit 1644
September 8, 2014

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/335,518	12/22/2011	Leonard Bell	ALXN-P07-114	4891

959 7590 02/06/2014

NELSON MULLINS RILEY & SCARBOROUGH LLP
FLOOR 30, SUITE 3000
ONE POST OFFICE SQUARE
BOSTON, MA 02109

EXAMINER	
GAMBEL, PHILLIP	
ART UNIT	PAPER NUMBER
1644	
MAIL DATE	DELIVERY MODE
02/06/2014	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. 13/335,518	Applicant(s) BELL ET AL.	
	Examiner PHILLIP GAMBEL	Art Unit 1644	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 *131-151*.

☐ 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) *131-151* is/are pending in the application.

5a) Of the above claim(s) *134,135,139,140,144,145,150 and 151* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *131-133,136-138,141-143 and 146-149* 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 *12/22/2011* 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 _____.

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

4) ☐ Other: _____.

DETAILED ACTION

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

2. Applicant's amendment, filed 12/09/2013, has been entered.

Claims 131-133, 136, 137, 141, 142 and 146-149 have been amended.

Claims 131-151 are pending.

Claims 1-130 have been canceled previously.

3. Applicant's election of the species (A) (methods of administering anti-C5 antibody, where the claimed do not comprise further administering one or more compounds that increase hematopoiesis in the Response to Restriction Requirement, filed 12/09/2013, is acknowledged.

Applicant's remarks concerning entitlement of claims to additional species upon allowance of a generic linking claim are acknowledged.

Claims 131-133, 136-138, 141-143 and 146-149 are under consideration in the instant application as they read on the elected species.

Claims 134, 135, 139, 140, 144, 145, 150 and 151 have been withdrawn as being drawn to the non-elected species.

4. Priority.

A) Applicant should update the status of the priority documents on page 1 of the specification.

USSN 12/902,617 is now abandoned.

B) Note that not all claims may receive benefit of the priority applications.

The recitation of "comprising administering to a patient with PNH an anti-C5 antibody at least once every 12 days to thereby maintain in the patient a serum complement activation at a level below 20% of the normal serum complement activity" and "at least once every 12 days" (e.g., see claims 136 and dependent claims thereof, claims 141 and dependent claims thereof).

Applicant's preliminary amendment filed 12/22/2011, directs support for the newly added claims to various pages of the specification but does not provide clear direct support for the above-mentioned limitations encompassing administration regimens that includes the recitation of "at least once every 12 days".

The written support of administration regimen that includes the recitation of "at least once every 12 days" is not readily apparent in the specification as-filed.

It is noted that a generic or a sub-generic disclosure cannot support a species unless the species is specifically described. It cannot be said that a subgenus is necessarily described by a genus encompassing it and a species upon which it reads. See In re Smith 173 USPQ 679, 683 (CCPA 1972) and MPEP 2163.05.

If applicant desires priority for the above-mentioned limitation / current claims prior to filing date of the instant application (12/22/2011);
applicant is invited to point out and provide documentary support for the priority of the instant claims.

Applicant is reminded that such priority for the instant limitations requires written description and enablement under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph.

Therefore, this application repeats a substantial portion of prior USSN 12/902,617, filed 10/12/2010 and adds and claims additional disclosure not presented in the prior application, as indicated above.
Since this application names an inventor or inventors named in the prior application, it may constitute a continuation-in-part of the prior application.

Should applicant desire to obtain the benefit of the filing date of the prior application, attention is directed to 35 U.S.C. 120 and 37 CFR 1.78.

Therefore, applicant should amend the first line of the specification to indicate the status of the instant application as a continuation-in-part and file an Application Data Sheet reflecting the proper relationship of the instant application to its priority documents.

A claim as a whole has only one effective filing date.
See Studiengelsellschaft Kahle m.b.H. v. Shell Oil Co. 42 USPQ2d 1674, 1677 (Fed. Cir 1997).

5. The application is required to be reviewed and all spelling, TRADEMARKS, and like errors corrected. Appropriate corrections are required.

Trademarks should be capitalized or accompanied by the ® or ™ symbol wherever they appear and be accompanied by the generic terminology. Although the use of trademarks is permissible in patent applications, the proprietary nature of the trademarks should be respected and every effort made to prevent their use in any manner, which might adversely affect their validity as trademarks.

The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP 608.01.

For example, see page 40 of the specification.

Appropriate corrections are required

6. 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.

7. Claims 131-133, 136-138, 141-143 and 146-149 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claims 131-133, 136-138, 141-143 and 146-149 are indefinite in the recitation of the "wherein clause" in the independent claims ("wherein the patient has experienced complement activity breakthrough on a dosing schedule of eculizumab comprising: (i) 600 mg of eculizumab each week for four weeks; (ii) 900 mg of eculizumab on the fifth week; and thereafter, (iii) 900 mg of eculizumab once every two weeks, and wherein the anti-C5 antibody inhibits the cleavage of complement component C5 into fragments C5a and C5b") because it is not clear as to whether the wherein clause defines positive steps in the claimed methods, defines specific patient populations (e.g., to be treated or have already been treated) or defines other characteristics of the claimed methods of elements recited within the claimed methods (e.g., characteristics of the anti-C5 antibody or eculizumab, capabilities / possibilities of the claimed methods).

Given the recitation of "wherein the patient has experienced complement activity breakthrough on a dosing schedule",

it is unclear whether the "wherein clause" necessarily recites any additional active method steps, but simply states a characterization or conclusion of the results of those steps or the patient populations that have been treated of possibly the characterization of the anti-C5 antibodies / eculizumab.

Note that the claim recites "wherein the patient has experienced complement activity breakthrough on a dosing schedule" in the past tense.

It is not clear whether or how the "wherein clause" further limits the claimed methods of treating PNH.

The intent, meaning and the metes and bounds of the claimed methods in view of the "wherein clause" are ambiguous and ill-defined.

The claims are 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.

Applicant is reminded that the amendment must point to a basis in the specification so as not to add any new matter. See MPEP 714.02 and 2163.06

8. The following is a quotation of 35 U.S.C. 112(a):
(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), first paragraph:
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

9. The specification is objected to as failing to provide proper antecedent basis for the claimed subject matter. See 37 C.F.R. § 1.75(d)(1) and M.P.E.P. § 608.01(o). Correction of the following is required:

The recitation of "comprising administering to a patient with PNH an anti-C5 antibody at least once every 12 days to thereby maintain in the patient a serum complement activation at a level below 20% of the normal serum complement activity" and "at least once every 12 days" (e.g., see claims 136 and dependent claims thereof, claims 141 and dependent claims thereof).

Applicant's preliminary amendment filed 12/22/2011, directs support for the newly added claims to various pages of the specification but does not provide clear direct support for the above-mentioned limitations encompassing administration regimens that includes the recitation of "at least once every 12 days".

The written support of administration regimen that includes the recitation of "at least once every 12 days" is not readily apparent in the specification as-filed.

Applicant is required to amend the specification to provide proper antecedent basis of claims 136 and dependent claims thereof and claims 141 and dependent claims thereof in the specification.

Alternatively, applicant is invited to identify the written support for instant claims in the specification as filed.

10. This is a written description rejection, new matter under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph.

Claims 131-133, 136-138, 141-143 and 146-149 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

The specification as originally filed does not provide support for the invention as now claimed:

The specification as originally filed does not provide support for the invention as now claimed:
"at least 35 μg/ml" and "wherein the patient has experienced complement activity breakthrough on a dosing schedule".

Applicant's amendment, filed 12/09/2013, directs support for the newly amended claims to paragraphs [0012], [0081], [0088] and [0093] of the corresponding US2012/0308559 and asserts no new matter has been filed.

However the specification as-filed appears to provide written description of "approximately 35 μg/ml to 50 μg/ml" and not an open-ended range of "at least 35 μg/ml" as currently recited (see pages 6-7, overlapping paragraph of the specification' paragraph [0088] of US2012/0308559).

Also, the specification as-filed appears to provide written description of "serum hemolytic activity" and not any/all "complement activity" as currently recited (see pages 6-7, overlapping paragraph of the specification' paragraph [0088] of US2012/0308559).

The newly amended "limitations" have broaden the written description of the Examples relied upon for the claimed "limitations" and, in turn, represent a departure of the specification / application as-filed.

The specification does not provide sufficient blazemarks nor direction for the instant methods encompassing the above-mentioned "limitations" as currently recited. The instant claims now recite limitations which were not clearly disclosed in the specification as-filed, and now change the scope of the instant disclosure as-filed. Such limitations recited in the present claims, which did not appear in the specification, as filed, introduce new concepts and violate the description requirement of the first paragraph of 35 U.S.C. 112.

Applicant is required to cancel the new matter in the response to this Office Action.

Alternatively, applicant is invited to provide sufficient written support for the "limitations" indicated above.
See MPEP 714.02 and 2163.06.

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

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

12. 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. §103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. § 103(c) and potential 35 U.S.C. §§ 102(f) or (g) prior art under 35 U.S.C. § 103(a).

13. Claims 131-133, 136-138 and 146-149 are rejected under 35 U.S.C. § 102(a)(b) as being anticipated by Hillmen et al. (Blood 102: 1858, 2003) (1449) (see entire document).

Since the public availability of this document is not clear and given that certain claims are subject to a rejection under 35 USC 112, first paragraph, written description / new matter; this rejection is being applied under 35 U.S.C. § 102(a)(b).

Hillmen et al. teach methods of treating paroxysmal nocturnal hemoglobulinuria (PNH), intravascular hemolysis and venous thrombosis, with 900 mg eculizumab by i.v. infusion every other week for one year an anti-C5 antibody and completing 11 and 16 months of therapy and in certain instances with erythropoietin as well, including improvement in biochemical / biological parameters associated with LDH, PNH type III cells and complement activity (see entire document).

The prior art dosage administration regimen reads on the claimed methods.

Consistent with the rejection under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, above and with respect to claim construction for prior art rejections, the following is noted.

Regarding the interpretive "wherein" clause recited in independent claims, the wherein clause does not necessarily recite any additional active method steps, but simply states a characterization or conclusion of the results of those steps or the patient populations that have been treated of possibly the characterization of the anti-C5 antibodies / eculizumab.

Therefore, the "wherein clause" is not considered to further limit the method defined by the claim and has been given its proper weight in construing the claims of administering to a patient with PNH an anti-C5 antibody in an amount and with a frequency effective to maintain a serum concentration of at least 35 μg/ml.
It does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure

Although the reference is silent about certain specific claimed limitations per se of a patient having paroxysmal nocturnal hemoglobulnuria (PNH) (e.g., see claims 133, 138, 149) or effective amounts to maintain serum complement activity at a certain range (e.g., see claims 131, 136, 146), it does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure. See Bristol-Myers Squibb Company v. Ben Venue Laboratories 58 USPQ2d 1508 (CAFC 2001). "{i}t is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable". In re Woodruff, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990). The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979).

Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145.

Given the prior art teaching of treating with 900 mg of eculizumab every other week, the prior art teaches effective amounts of treatment with the anti-C5 antibody eculizumab occurs in a dosing regimen at least once every 12 days.

On this record, it is reasonable to conclude that the same PNH patients are being administered the same active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., LDH, complement activity and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,
it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to administer anti-C5 antibodies to treat PNH patients.

14. Claims 131-133, 136-138 and 146-149 are rejected under 35 U.S.C. § 102(b) as being anticipated by Hillmen (44th ASH Annual Meeting Abstract, Blood, Vol. 100, 2002) (1449) (see entire document).

Hillmen (2002) teaches that paroxysmal nocturnal hemoglubinuria (PNH) is characterized by intravascular hemolysis and venous thrombosis. Hillmen teaches that the hemolysis is complement-mediated and due to the deficiency of complement regulatory proteins on red cells, particularly the terminal complement inhibitor CD59 (see entire document). Hillmen teaches treating transfusion-dependent PNH patients with 600 mg weekly for four (4) doses and 900 mg every other week of ECULIZUMAB, a humanized anti-C5 antibody (see entire publication). Hillmen teaches that the biological parameters of hemolysis dramatically improved or normalized for all patients within a week of starting therapy. Hillmen further teaches that mean LDH decreased greater than 50%, from 4494 +/- 3380 units/liter to 636 +/- 166 units/liter.

The prior art dosage administration regimen reads on the claimed methods

Consistent with the rejection under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, above and with respect to claim construction for prior art rejections,
the following is noted.

Regarding the interpretive "wherein" clause recited in independent claims,
the wherein clause does not necessarily recite any additional active method steps, but simply states a characterization or conclusion of the results of those steps or the patient populations that have been treated of possibly the characterization of the anti-C5 antibodies / eculizumab.

Therefore, the "wherein clause" is not considered to further limit the method defined by the claim and has been given its proper weight in construing the claims of administering to a patient with PNH an anti-C5 antibody in an amount and with a frequency effective to maintain a serum concentration of at least 35 μg/ml.
It does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure

Although the reference is silent about certain specific claimed limitations per se of a patient having paroxysmal nocturnal hemoglobulnuria (PNH) (e.g., see claims 133, 138 and 149) or effective amounts to maintain serum complement activity at a certain range (e.g., see claims 131, 136 and 146),
it does not appear that the claim language or limitations result in a manipulative difference in the method steps when compared to the prior art disclosure. See Bristol-Myers Squibb Company v. Ben Venue Laboratories 58 USPQ2d 1508 (CAFC 2001). "{i}t is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable". In re Woodruff, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990). The mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious. Mere recognition of latent properties in the prior art does not render nonobvious an otherwise known invention. In re Wiseman, 201 USPQ 658 (CCPA 1979). Granting a patent on the discovery of an unknown but inherent function would remove from the public that which is in the public domain by virtue of its inclusion in, or obviousness from, the prior art. In re Baxter Travenol Labs, 21 USPQ2d 1281 (Fed. Cir. 1991). See M.P.E.P. 2145.

Given the prior art teaching of treating with 900 mg of eculizumab every other week,
the prior art teaches effective amounts of treatment with the anti-C5 antibody eculizumab occurs in a dosing regimen at least once every 12 days.

On this record, it is reasonable to conclude that the same PNH patients are being administered the same active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., LDH, complement activity and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,

it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to administer anti-C5 antibodies to treat PNH patients.

15. Claims 131-133, 136-138, 141-143 and 146-149 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hillmen et al. (Blood 102: 1858, 2003) (1449) AND/OR Hillmen (44th ASH Annual Meeting Abstract, Blood, Vol. 100, 2002) (1449) in view of Meyers et al. (Int. J. Hematol. 77: 125-132, 2003) (1449) and as evidenced by Garba et al. (Singapore Med. J. 46(11):632-634, 2005) (1449) with respect to normal LDH levels.

Hillmen et al. (2003) teach methods of treating paroxysmal nocturnal hemoglobulinuria (PNH), intravascular hemolysis and venous thrombosis, with 900 mg eculizumab by i.v. infusion every other week for one year an anti-C5 antibody and completing 11 and 16 months of therapy and in certain instances with erythropoietin as well, including improvement in biochemical / biological parameters associated with LDH, PNH type III cells and complement activity (see entire document).

Hillmen (2002) teaches that paroxysmal nocturnal hemoglubinuria (PNH) is characterized by intravascular hemolysis and venous thrombosis. Hillmen teaches that the hemolysis is complement-mediated and due to the deficiency of complement regulatory proteins on red cells, particularly the terminal complement inhibitor CD59 (see entire document). Hillmen teaches treating transfusion-dependent PNH patients with 600 mg weekly for four (4) doses and 900 mg every other week of ECULIZUMAB, a humanized anti-C5 antibody (see entire publication). Hillmen teaches that the biological parameters of hemolysis dramatically improved or normalized for all patients within a week of starting therapy. Hillmen further teaches that mean LDH decreased greater than 50%, from 4494 +/- 3380 units/liter to 636 +/- 166 units/liter.

It does not appear that the claim language or limitations result in an unobvious manipulative difference in the method steps when compared to the prior art disclosure of providing 600 mg weekly for four (4) doses and 900 mg every other week of eculizumab a humanized anti-C5 antibody and maintenance doses in the treatment of patients with PNH at the time the invention was made.

Given the prior art teachings of treating with 900 mg of eculizumab every other week,

the prior art teaches effective amounts of treatment with the anti-C5 antibody eculizumab occurs in a dosing regimen at least once every 12 days.

On this record, it is reasonable to conclude that the same PNH patients are being administered the same active agent anti-C5 antibodies by the same mode of administration in the same amount to achieve the same or nearly the same improvement in biochemical and biological parameters (e.g., LDH, complement activity and PNH type III cells) in both the instant claims and the prior art reference. The fact that applicant may have discovered yet another beneficial effect from the method set forth in the prior art does not mean that they are entitled to receive a patent on that method.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,

it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Further, the mean level of LDH in the PNH patients was more than 1.5 times the upper limit of a normal LDH level in a person, as evidenced by Garba, who discloses that normal LDH in a person is 247.10 +/- 19.0 units/liter (Abstract in particular). The prior art teaching clearly anticipates the claimed invention.

Regarding the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations" recited in the claims,

it is noted that these limitations do not recite any additional active method steps, but simply states a characterization, an intended result or a conclusion of a process step or patient populations positively recited.

Meyers et al. teach management issues in the treatment of paroxysmal nocturnal hemoglobinuria (see entire document), including the hallmarks of hemolysis and anemia (e.g., see pages 125-128) as well as the treatment of this syndrome with complement inhibitors such as anti-C5 antibodies particularly for the hemolysis of PNH (see Section 2.5, Complement Inhibitors) and erythropoietin (see Section 2.6, Management of Nonhemolytic Anemia).

In addition, as to the "frequencies to maintaining serum concentrations of antibodies" and "patient characterizations",

treating PNH patients characterized by the criteria of the proportion of type III red blood cells, platelet counts, type III granulocyte clone and serum complement activity are result effective variables of controlling hemolysis and reducing the risk of venous thrombosis with anti-C5 antibodies in the treatment of PNH patient populations who are characterized by intravascular hemolyisis, venous thrombosis and aplastic anemia.

It is well settled that "discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art." In re Boesch, 617 F.2d 272, 276, 205 USPQ 215, 219 (CCPA 1980). See also Merck & Co. v. Biocraft Labs. Inc., 874 F.2d 804, 809, 10 USPQ2d 1843, 1847-48 (Fed. Cir. 1989) (determination of suitable dosage amounts in diuretic compositions considered a matter of routine experimentation and therefore obvious).

As the nature of the dosing and therapeutic endpoints were known to the ordinary artisan, it would have been obvious to optimize the frequency of anti-C5 antibodies in order to control hemolysis and the risk of venous thrombosis in a variety of PNH patients.

Given the nature of the clinical manifestations of PNH such as hemolysis and anemia, one of ordinary skill in the art at the time the invention was made would have been motivated to select and combine both anti-C5 antibodies and erythropoietin to address and manage the clinical manifestations of PNH over time. One would have been motivated to combine these therapies with a reasonable expectation of success because each treats a different aspect of the hemolytic disease in the patient and could be reasonably expected to improve the prognosis of the patient more rapidly than either therapy alone. From the teachings of the references, it was apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

"The test of obviousness is not express suggestion of the claimed invention in any or all of the references but rather what the references taken collectively would suggest to those of ordinary skill in the art presumed to be familiar with them." See In re Rosselet, 146 USPQ 183, 186 (CCPA 1965).

"There is no requirement (under 35 USC 103(a)) that the prior art contain an express suggestion to combine known elements to achieve the claimed invention. Rather, the suggestion to combine may come from the prior art, as filtered through the knowledge of one skilled in the art." Motorola, Inc. v. Interdigital Tech. Corp., 43 USPQ2d 1481, 1489 (Fed. Cir. 1997).

An obviousness determination is not the result of a rigid formula disassociated from the consideration of the facts of a case. Indeed, the common sense of those skilled in the art demonstrates why some combinations would have been obvious where others would not. See KSR Int'l Co. v. Teleflex Inc., 82 USPQ2d 1385 (U.S. 2007) ("The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.").

16. Given copending applications encompassing similar subject matter and the restriction set forth herein, applicant is invited to clarify which applications should be subject to rejections under the judicially created doctrine of obviousness-type double patenting.

17. No claim is allowed.

18. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (571) 272-0844. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached on (571) 272-3181.

The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Phillip Gambel/

Primary Examiner
Technology Center 1600
Art Unit 1644
February 4, 2014

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/335,518	12/22/2011	Leonard Bell	ALXN-P07-114	4891

28120 7590 11/07/2013

ROPES & GRAY LLP
IPRM - Floor 43
PRUDENTIAL TOWER
800 BOYLSTON STREET
BOSTON, MA 02199-3600

EXAMINER	
GAMBEL, PHILLIP	
ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
11/07/2013	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):

USPatentMail@ropesgray.com
USPatentMail2@ropesgray.com

Office Action Summary	Application No. 13/335,518	Applicant(s) BELL ET AL.	
	Examiner PHILLIP GAMBEL	Art Unit 1644	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 *1* 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 *12/22/2011, 10/03/2012*.

☐ 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) *131-151* 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) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *131-151* 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 _____.

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

4) ☐ Other: _____.

DETAILED ACTION

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

2. Applicant's amendment, filed 12/22/2011, has been entered.
Claims 1-130 have been canceled.
Claims 131-145 have been added.

Applicant's amendment, filed 10/03/2012, has been entered.
Claim 131 has been amended.
Claims 146-151 have been added.

Claims 131-151 are pending.

3. Applicant is required to elect
A) whether the claimed method do NOT comprise further administering one or more compounds that increase hematopoiesis **OR**
B) whether the claimed method do comprise further administering one or more compounds that increase hematopoiesis.

If applicant elects (B),
then applicant is required to elect a specific one or more compound that increase hematopoiesis from those recited in claims 135, 140 and 145 and more particularly from pages 8-9, 12 and 21-22 of the instant specification.
If applicant elects "one or more compounds", then applicant should clearly define the one or more compounds *(e.g., iron, antithymocyte globulin, cyclosporine, ATG, EPO derivatives, etc.).*

The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

These species of (A) and (B) are distinct because they rely upon different ingredients, methods steps and endpoints.

In turn, the steps rely upon ingredients and compounds that differ in their structures, physicochemical properties and modes of action that differ action to such an extent and require non-coextensive searches to such an extent that they are considered patentable distinct. Further, these ingredients and compounds do not share a substantial structural feature essential to a common utility do not have common structure to a common utility. Therefore, they are separate and patentably distinct species

These species of compounds are distinct because their structures, physicochemical properties and modes of action differ action to such an extent and require non-coextensive searches to such an extent that they are considered patentable distinct. Further, these compounds do not share a substantial structural feature essential to a common utility do not have common structure to a common utility. Therefore, they are separate and patentably distinct species.

Applicant is required under 35 U.S.C. § 121 to elect a single disclosed species (*e.g., A or B and if B, then specify one or more compounds)* for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 131, 136 and 141 are generic, for example.

There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

4. Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected species, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election of the species may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species.

Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

5. Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

6. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phillip Gambel whose telephone number is (571) 272-0844. The examiner can normally be reached Monday through Thursday from 7:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached on (571) 272-3181.

The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Phillip Gambel/
Primary Examiner
Technology Center 1600
Art Unit 1644
November 4, 2013

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

133485 7590 08/16/2016

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
08/16/2016	ELECTRONIC

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

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

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

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 15/148,839	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	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* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

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

Status

1)☒ Responsive to communication(s) filed on *5/6/2016*.

☐ 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) *1* 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) *1* 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/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *5/6/2016*.

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

4) ☐ Other: _____.

DETAILED ACTION

1. Claim 1 is pending and under consideration in the instant examination and reads on a compositions which is a 300 mg eculizumab single-use dosage form comprising 30 ml of a 10 mg eculizumab/ml sterile, preservative free solution.

2. Applicant's IDSs, filed on 05/06/2016, are acknowledged and have been considered.

Priority

3. Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of 35 U.S.C. 112(a) or the first paragraph of 35 U.S.C. 112 (pre-AIA). See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosure of the prior-filed application, US Provisional Application No. 60/783,070, fails to provide adequate support or enablement in the manner provided by 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph for one or more claims of this application. In particular, the claim recites a composition which is a 300 mg eculizumab single-use dosage comprising 30 ml of a 10 mg eculizumab/ml steril, preservative free solution.

Accordingly, the priority date of the instant claims of the current application is considered to be **3/15/2007**, the filing date of PCT/US2007/006606.

Claim Rejections - 35 USC § 103

4. 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 even 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

5. Claim 1 is rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 552-9), in view of Evans et al. (US 6,355,245 B1, issued 3/12/2002), Wang (US 2005/0271660), as is evidenced by the parent specification of US Patent Application No. 13/426,973 at p. 16, lines 20-25.

Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶). Hillmen teaches that patients received infusions of eculizumab weekly for four weeks, followed one week later by a 900 mg dose and then by a dose of 900 mg every other week through week 12 (p. 554 under “treatment schedule”).

The prior art references differ from the claimed invention in the recitation that the composition is a 300 mg eculizumab single-use dosage form comprising 30 ml of a 10 mg eculizumab/ml steril, preservative free solution.

However, Evans teaches antibody 5G1.1, which is the same as eculizumab (see parent specification US Patent Application No. 13/426,973, p. 16 lines 20-25), is effective at doses of 2.2, 3, or 5 mg per kg body weight; this corresponds to 154, 210, or 350 mg of antibody for a 70kg adult human (‘245 patent, column 16 lines 10-30). Evans also teaches that eculizumab is therapeutically effective when as little as 210 mg are present (column 16 lines 10-15). Evans teaches that the antibodies are particularly advantageous in that they are highly active even when administered in low doses (col 8 lines 25-32), providing a reasonable expectation of success in treating PHN with a dose less than what was disclosed by Hillmen. The reference teaches that it is within the skill of an artisan, particularly a physician, to determine the optimal dose (column 18 lines 15-30). Evans teaches formulations of the anti-C5 antibodies may be formulated in a pharmaceutically effective carrier such saline, which is considered to be a "preservative free solution". (col 18 lines 30-45).

However neither Hillmen nor '245 teaches a 300 mg single unit dosage as recited in the claims.

Nevertheless it would have been obvious to one of ordinary skill in the art to modify the teachings of Hillmen to administer 300 mg single unit dosages. Hillmen teaches that both 600 and 900 mg dosages can be used, and since Evans patent teaches that 210 or 350 mg of antibody would be expected to be therapeutically effective, arriving at the 300 mg dosage would be routine, convenient and obvious. One of ordinary skill in the art would have a reasonable expectation of success, as Evans teaches that the antibody exerts its effect at low doses.

Finally, Wang teaches that eculizumab is highly soluble, including at up to 30 mg per ml (paragraph 0171, Figure 10). Wang further teaches that pharmaceutically acceptable carriers include pyrogen-free water may be used in formulation of the antibody (¶71) (sterile, preservative free solution). Therefore the artisan of ordinary skill would have had a reasonable expectation of success in making a 10 mg / ml solution of the antibody sterile, preservative free solution.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

6. No claim is allowed.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

August 9, 2016

/JAMES ROGERS/

Examiner, Art Unit 1644

/SHARON WEN/
Primary Examiner, Art Unit 1644

Eculizumab: 5G1.1, h5G1.1, Long-Acting Anti-C5 Monoclonal Antibody 5G1-1, Long-Acting Anti-C5 Monoclonal Antibody 5G1.1

Author: Adis International Limited,

Publication info: Drugs in Research and Development 8.1 (2007): 61-68.

ProQuest document link

Abstract (English): Eculizumab [long-acting anti-C5 monoclonal antibody 5G1- 1, 5G1.1, h5G1.1, monoclonal antibody 5G1.1, Soliris] is a monoclonal antibody that binds to the C5 complement molecule, thereby blocking the progression of the complement cascade at this point. By binding to C5, eculizumab prevents generation of the potent anaphylatoxin C5a and the cytolytic C5b-9 complex, or membrane attack complex. The compound has been filed for approval in the US and the EU for paroxysmal nocturnal haemoglobinuria (PNH), a chronic blood disease caused by a defective enzyme that leads to a lack of protective natural complement inhibitors. It is also being investigated for the treatment of asthma and transplantation. Eculizumab is a long- acting, humanised version of the anti-C5 antibody [h5G1.1]. The short-acting version is called pexelizumab. In January 2003 Alexion Pharmaceuticals executed a large-scale product supply agreement with Lonza Biologics for the long-term commercial manufacture of eculizumab. Specific terms of the agreement were not disclosed. Alexion signed an agreement with GTC Biotherapeutics in 2000 for the production of eculizumab in transgenic goats. However, this agreement no longer appears to be active. Alexion has also filed for approval for eculizumab in PNH in the EU under the centralised procedure. The EMEA has granted Alexions request for evaluation of the proposed MAA for eculizumab in PNH under the accelerated assessment procedure. This shortens the review part of the MAA procedure. Results from the phase III TRIUMPH trial have been reported; all pre- specified primary and secondary endpoints were achieved with statistical significance. The last patient completed the last visit in the SHEPARD trial in October 2006, and 12-month data is being submitted to regulatory applications. Positive preliminary interim results of 6 months of therapy have been reported. The study has been conducted to collect additional data on eculizumabs safety among haemolytic PNH patients who have undergone transfusion. The trial enrolled 97 patients in the US, Canada, Europe and Australia and includes 12 months of treatment. A pilot, open-label phase Ib trial evaluating eculizumab in 11 patients with PNH has been completed in the UK. All 11 patients in the trial chose to participate in two consecutive 1-year extension studies. One of these is still ongoing, with patients having completed at least 26 months of chronic eculizumab therapy. One- and two-year cumulative results have been presented. The positive findings further supported progression of the clinical development of eculizumab for PNH. Eculizumab has been granted orphan drug status in the US and EU for the treatment of PNH. . Phase I and II trials in rheumatoid arthritis had previously been completed. The rationale behind evaluating eculizumab in this indication was that activated terminal complement proteins appear to have a central role in the pathogenesis of immune-mediated joint inflammation, indicating that anti-C5 monoclonal antibodies may have therapeutic potential for the treatment of rheumatoid arthritis. A phase I pilot trial was completed in patients with dermatomyositis, an inflammatory skin and muscle disorder, for which eculizumab has orphan drug status in the US. The US FDA granted eculizumab fast-track designation in membranous glomerulonephritis in early 2000 and the drug has orphan drug status in the US for idiopathic membranous glomerular nephropathy. Both long-acting and short-acting versions of anti- C5 monoclonal antibody 5G1.1 are being developed by Alexion Pharmaceuticals in the US, with the use of technology licensed from Stanford University. Eculizumab is covered by US Patent No. 6 355 245, which extends to C5-specific monoclonal antibodies for the treatment of inflammatory diseases. The Japanese Patent Office issued patent number 3 734 266 for eculizumab in Japan in February 2006. The patent includes the use of eculizumab to inhibit complement activation in humans, the key mechanism of action used for the treatment of PNH. Corresponding patents have been issued or are pending in Europe, Canada, Australia and other key markets.

Subject: Statistics;Joint diseases;Membrane attack complex;Transfusion;Clinical trials;Dermatomyositis;Inflammatory diseases;Nephropathy;Drugs;Anaphylatoxin C5a;Skin;Membranous glomerulonephritis;Monoclonal antibodies;Patents;Muscles;Asthma;Enzymes;complement component C5;Drug development;Inflammation;Blood;Rheumatoid arthritis;Complement activation;Pharmaceuticals;Complement inhibitors

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Alexion completes enrollment in Ph II kidney disease trial

Publication info: Marketletter (Feb 25, 2002): NA.
ProQuest document link

Abstract (English): Alexion has completed enrollment in a Phase II study of its complement C5 inhibitor eculizumab (formerly 5G1.1) in patients with the kidney disease membranous nephritis. The drug has just been granted orphan status in the USA for this indication.

Full text: Alexion has completed enrollment in a Phase II study of its complement C5 inhibitor eculizumab (formerly 5G1.1) in patients with the kidney disease membranous nephritis. The drug has just been granted orphan status in the USA for this indication.

Subject: Drugs

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.

Identifier (keyword): Trade, ALXN

Event classification: 331: Product development

NAICS classification: 325412: Pharmaceutical Preparation Manufacturing

Product classification: 2834000: Pharmaceutical Preparations

SIC classification: 2834: Pharmaceutical preparations

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ISSN: 0951-3175

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Language of publication: English

Document type: Brief Article

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Alexion Completes Enrollment in Phase II Membranous Nephritis Clinical Trial with 5G1.1; - 5G1.1 Receives Orphan Drug Designation in Membranous Nephritis -

Publication info: PR Newswire 20 Feb 2002: NA.

ProQuest document link

Abstract (English): CHESHIRE, Conn. -- Alexion Pharmaceuticals Inc. announced today that it has completed enrollment in its first Phase II trial in patients suffering from a severe form of kidney disease known as membranous nephritis, with its anti-inflammatory C5 Inhibitor antibody, 5G1.1, now called eculizumab. Separately, Alexion announced that it has received orphan drug designation from the Food and Drug Administration for the use of eculizumab, if approved, in patients with membranous nephritis.
This Phase II multi-center, double-blinded, randomized, placebo-controlled study examines the safety and clinical efficacy of repeated dosing of eculizumab during a four month treatment period. Approximately 115 membranous nephritis patients were enrolled in the United States.

Full text: CHESHIRE, Conn. -- Alexion Pharmaceuticals Inc. announced today that it has completed enrollment in its first Phase II trial in patients suffering from a severe form of kidney disease known as membranous nephritis, with its anti-inflammatory C5 Inhibitor antibody, 5G1.1, now called eculizumab. Separately, Alexion announced that it has received orphan drug designation from the Food and Drug Administration for the use of eculizumab, if approved, in patients with membranous nephritis.

This Phase II multi-center, double-blinded, randomized, placebo-controlled study examines the safety and clinical efficacy of repeated dosing of eculizumab during a four month treatment period. Approximately 115 membranous nephritis patients were enrolled in the United States. Alexion also announced that, as a part of its comprehensive development plan for eculizumab, it has already commenced dosing in a follow-on open label extension study of eculizumab in these patients, designed to examine long-term safety and tolerability.

"Completing enrollment in the first eculizumab Phase II trial in patients suffering from membranous nephritis brings us closer to our objective of expanding the profile of eculizumab into a variety of chronic autoimmune opportunities," stated Leonard Bell, M.D., President and Chief Executive Officer of Alexion. "Once the four month treatment period and all follow-up patient visits are completed, we expect to complete data collection and then lock the database for subsequent analysis of this Phase II trial. Additionally, we are encouraged by the orphan drug designation as it may provide an innovative therapy for this small, but underserved patient population, while potentially providing us with development and commercial advantages."

According to the National Kidney Foundation, more than 260,000 Americans suffer from chronic kidney failure, and more than 50,000 people die each year because of kidney disease. Membranous nephritis is the most common cause of the nephrotic syndrome in adults worldwide, and is characterized by the abnormal excretion of large amounts of protein in the urine (proteinuria), which can cause a number of severe medical complications. Membranous nephritis is a form of kidney disease that is believed to be caused by a chronic autoimmune disorder in which antibodies and complement target the kidney. It is estimated that there are approximately 100,000 - 200,000 people currently afflicted with idiopathic membranous nephritis in the United States. Currently, in such a severe disease population, more than 30% of the patients are expected to progress to renal failure, which may require dialysis or transplantation.

Alexion is engaged in the discovery and development of therapeutic products aimed at treating patients with a wide array of severe disease states, including cardiovascular and autoimmune disorders, inflammation and cancer. Alexion's two lead product candidates, pexelizumab and 5G1.1, are currently in eight clinical development programs. Alexion is developing pexelizumab, an antibody fragment, in collaboration with Procter &Gamble Pharmaceuticals. Together the firms have initiated a Phase III clinical study with pexelizumab in cardiopulmonary bypass patients, and are currently conducting two large Phase II studies with pexelizumab in

acute myocardial infarction patients. Alexion's other lead product candidate, 5G1.1, has completed a Phase II trial for the treatment of rheumatoid arthritis. 5G1.1 is also in Phase II trials for the treatment of membranous nephritis and for lupus nephritis, and in earlier stage clinical trials for the treatment of dermatomyositis and pemphigoid. Additionally, through its wholly owned subsidiary, Alexion Antibody Technologies, Inc., Alexion is engaged in discovering and developing a portfolio of additional antibody therapeutics targeting severe unmet medical needs. This press release and further information about Alexion Pharmaceuticals, Inc. can be found on the World Wide Web at: http://www.alexionpharm.com/.

This news release contains forward-looking statements. Such statements are subject to certain factors which may cause Alexion's plans to differ or results to vary from those expected, including the results of pre-clinical or clinical studies (including termination or delay in clinical programs or inability to move forward to the next Phase of clinical development), the need for additional research and testing, delays in developing or arranging satisfactory manufacturing capability, inability to access capital and funding on a timely basis and on favorable terms, delays in development of or adverse changes in status of commercial relationships, the possibility that favorable results of earlier clinical trials are not predictive of safety and efficacy results in larger clinical trials, dependence on Procter &Gamble Pharmaceuticals for performance of development and commercial matters related to pexelizumab, the risk that third parties won't agree to license us on reasonable terms their intellectual property necessary for us to develop and commercialize our products, and a variety of other risks set forth from time to time in Alexion's filings with the Securities and Exchange Commission, including but not limited to the risks discussed in Alexion's Annual Report on Form 10-K for the year ended July 31, 2001. Except in special circumstances in which a duty to update arises under law when prior disclosure becomes materially misleading in light of subsequent events, Alexion does not intend to update any of these forward-looking statements to reflect events or circumstances after the date hereof or to reflect the occurrence of unanticipated events.

Contacts:
Alexion Pharmaceuticals, Inc.
Leonard Bell, M.D.
President &CEO
(203) 272-2596
Noonan/Russo Communications, Inc.
Ernie Knewitz (Media)
(212) 696-4455 Ext. 204
Rx Communications
Rhonda Chiger (Investor)
917-322-2569
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http://tbutton.prnewswire.com/prn/11690X36378205

Contact: Leonard Bell, M.D., President &CEO of Alexion Pharmaceuticals, Inc., +1-203-272-2596; media - Ernie Knewitz of Noonan-Russo Communications, Inc., +1-212-696-4455 Ext. 204; or investor - Rhonda Chiger of Rx Communications, +1-917-322-2569, both for Alexion Pharmaceuticals Inc.

Subject: Drugs;Pharmaceutical industry

Company / organization: Alexion Pharmaceuticals Inc.

Identifier (keyword): Trade, ALXN

NAICS classification: 325412: Pharmaceutical Preparation Manufacturing

Product classification: 2834000: Pharmaceutical Preparations

SIC classification: 2834: Pharmaceutical preparations

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Alexion issued C5 complement inhibitor patent for inflammatory diseases

Publication info: ESPICOM Pharmaceutical and Medical Device News (Mar 19, 2002).
ProQuest document link

Full text: Alexion Pharmaceuticals has been issued US Patent No. 6,355,245 entitled: "C5-Specific Antibodies for the Treatment of Inflammatory Diseases," from the US PTO. The issued claims relate to the company's C5 complement inhibitor programme and cover the composition and use of Alexion's lead drug candidates, eculizumab (formerly known as 5G1.1) and pexelizumab, as well as other antibodies that bind to C5 and effectively inhibit its inflammatory activity.

Subject: Pharmaceutical

Language: English

Document type: Article

Publication title: ESPICOM Pharmaceutical and Medical Device News

Publication type: Newsletter

Publication date: Mar 19, 2002

Source attribution: ESPICOM Pharmaceutical and Medical Device News, © Publisher specific

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First available: 2010-11-15

Database: ESPICOM Pharmaceutical &Medical Device News (2000 - current)

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Alexion Issued Key C5 Complement Inhibitor Patent for Inflammatory Diseases

Publication info: PR Newswire 15 Mar 2002: NYF00215032002.
ProQuest document link

Abstract (English): CHESHIRE, Conn. -- Alexion Pharmaceuticals, Inc. today announced that it has been issued United States Patent No. 6,355,245 entitled "C5-Specific Antibodies for the Treatment of Inflammatory Diseases," from the United States Patent and Trademark Office. The issued claims relate to the company's C5 Complement Inhibitor program and cover the composition and use of Alexion's lead drug candidates, eculizumab (formerly known as 5G1.1) and pexelizumab, as well as other antibodies that bind to C5 and effectively inhibit its inflammatory activity.
"Issuance of the patent further supports our aggressive pursuit to develop pexelizumab and eculizumab in a variety of severe acute and chronic inflammatory conditions and is an important element in our commercialization strategy," stated Leonard Bell, M.D., President and Chief Executive Officer of Alexion.

Full text: CHESHIRE, Conn. -- Alexion Pharmaceuticals, Inc. today announced that it has been issued United States Patent No. 6,355,245 entitled "C5-Specific Antibodies for the Treatment of Inflammatory Diseases," from the United States Patent and Trademark Office. The issued claims relate to the company's C5 Complement Inhibitor program and cover the composition and use of Alexion's lead drug candidates, eculizumab (formerly known as 5G1.1) and pexelizumab, as well as other antibodies that bind to C5 and effectively inhibit its inflammatory activity.
"Issuance of the patent further supports our aggressive pursuit to develop pexelizumab and eculizumab in a variety of severe acute and chronic inflammatory conditions and is an important element in our commercialization strategy," stated Leonard Bell, M.D., President and Chief Executive Officer of Alexion. "This patent represents the cornerstone of our proprietary position in C5 complement inhibition. It is also significant as it comes at a time when we have achieved several key milestones in our clinical programs including commencement of enrollment in the Phase III pivotal PRIMO-CABG trial and completion of enrollment in the Phase II COMPLY myocardial infarction trial with pexelizumab, and in parallel, the initiation of our first Phase IIb trial in rheumatoid arthritis and the completion of enrollment of the Phase II membranous nephritis trial with eculizumab."
Alexion is engaged in the discovery and development of therapeutic products aimed at treating patients with a wide array of severe disease states, including cardiovascular and autoimmune disorders, inflammation and cancer. Alexion's two lead product candidates, pexelizumab and eculizumab, are currently in eight clinical development programs. Alexion is developing pexelizumab, an antibody fragment, in collaboration with Procter &Gamble Pharmaceuticals. Together the firms have initiated a Phase III clinical study with pexelizumab in cardiopulmonary bypass patients, and are currently conducting two large Phase II studies with pexelizumab in acute myocardial infarction patients. Alexion's other lead product candidate, eculizumab, is being studied in a Phase IIb trial for the treatment of rheumatoid arthritis. Eculizumab is also in Phase II trials for the treatment of membranous nephritis and for lupus nephritis, and in earlier stage clinical trials for the treatment of dermatomyositis and pemphigoid. Additionally, through its wholly owned subsidiary, Alexion Antibody Technologies, Inc., Alexion is engaged in discovering and developing a portfolio of additional antibody therapeutics targeting severe unmet medical needs. This press release and further information about Alexion Pharmaceuticals, Inc. can be found on the World Wide Web at: http://www.alexionpharm.com/ .
This news release contains forward-looking statements. Such statements are subject to certain factors which may cause Alexion's plans to differ or results to vary from those expected, including the results of pre-clinical or clinical studies (including termination or delay in clinical programs or inability to move forward to the next Phase of clinical development), the need for additional research and testing, delays in developing or arranging

satisfactory manufacturing capability, inability to access capital and funding on a timely basis and on favorable terms, delays in development of or adverse changes in status of commercial relationships, the possibility that favorable results of earlier clinical trials are not predictive of safety and efficacy results in larger clinical trials, dependence on Procter &Gamble Pharmaceuticals for performance of development and commercial matters related to pexelizumab, the risk that third parties won't agree to license us on reasonable terms their intellectual property necessary for us to develop and commercialize our products, and a variety of other risks set forth from time to time in Alexion's filings with the Securities and Exchange Commission, including but not limited to the risks discussed in Alexion's Annual Report on Form 10-K for the year ended July 31, 2001. Except in special circumstances in which a duty to update arises under law when prior disclosure becomes materially misleading in light of subsequent events, Alexion does not intend to update any of these forward-looking statements to reflect events or circumstances after the date hereof or to reflect the occurrence of unanticipated events.

CONTACT:
Alexion Pharmaceuticals, Inc.
Stephen P. Squinto, Ph.D.
Executive Vice President and Head of Research
(203) 272-2596
Noonan/Russo Communications, Inc.
Ernie Knewitz (Media)
(212) 696-4455 Ext. 204
Rx Communications
Rhonda Chiger (Investor)
917-322-2569
MAKE YOUR OPINION COUNT - Click Here
http://tbutton.prnewswire.com/prn/11690X75229070

Contact: Stephen P. Squinto, Ph.D., Executive Vice President and Head of Research of Alexion Pharmaceuticals, +1-203-272-2596, or Media, Ernie Knewitz of Noonan-Russo Communications, +1-212-696-4455, ext. 204, or Investor, Rhonda Chiger of Rx Communications +1-917-322-2569
Website: http://www.noonanrusso.com/index.html

Subject: Drugs;Pharmaceutical industry

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.

Identifier (keyword): Trade, ALXN

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Product classification: 2834000: Pharmaceutical Preparations

SIC classification: 2834: Pharmaceutical preparations

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ALEXION OFFERS EARLY, MIXEDPHASE III DATA FOR HEART DRUG

Author: Osborne, Randall

Publication info: BIOWORLD Today 14.151 (Aug 6, 2003): NA.

ProQuest document link

Abstract (English): Byline: Randall Osborne , National Editor

Alexion Pharmaceuticals Inc. narrowly missed its primary endpoint in the preliminary analysis of its Phase III heart surgery drug, pexelizumab, but the company said other indicators are encouraging enough to ask the FDA about filing for approval.

But that didn't thrill Wall Street, which took away 21.8 percent from Alexion shares. Its stock (NASDAQ:ALXN) fell $3.49 Tuesday to close at $12.51, after dipping as low as $12.03.

Leonard Bell, CEO of Cheshire, Conn.-based Alexion, declined to estimate when the company will know its next step with the drug, but told BioWorld Today, "What we need to do is complete all of our internal diligence" and begin talks with the FDA.

Full text: Byline: Randall Osborne , National Editor

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Leonard Bell, CEO of Cheshire, Conn.-based Alexion, declined to estimate when the company will know its next step with the drug, but told BioWorld Today, "What we need to do is complete all of our internal diligence" and begin talks with the FDA.

Full data from the 3,000-patient trial, called "Pexelizumab for Reduction in Infarction and Mortality in Coronary Artery Bypass Graft Surgery," or the PRIMO-CABG trial, won't be disclosed until November at the annual meeting of the American Heart Association.

Embargo rules prevent Alexion from disclosing more about the Phase III results now, Bell said, but noted in a conference call there was "substantial internal consistency in the data set. We think that the sum total of the data of pexelizumab in CABG is supportive of having a clinically meaningful effect."

In the figures that could be disclosed, Alexion's short-acting, single- chain antibody C5 complement inhibitor failed to hit its primary endpoint: reducing death and myocardial infarction at 30 days in patients undergoing CABG who were not undergoing valve surgery at the same time.

Between 10 percent and 12 percent of CABG patients undergo valve surgery at the same time as their bypass graft procedure, but Alexion disqualified them from primary analysis of the trial since Phase II data suggested pexelizumab is less likely to work in that setting.

In the Phase III study, however, the drug did reach borderline statistical significance ($p<0.05$) for both sets of patients - those who underwent valve surgery and those who didn't - in reducing death and myocardial infarction. That "all comers" analysis made up one of the secondary endpoints.

Alexion will provide no further p values or scientific details until the
AHA meeting.

Eric Schmidt, analyst with SG Cowen Securities Corp., called the secondary endpoint finding "ironic" and meaningful in a favorable way. Bell agreed the Phase III outcome is ironic but said the choice to exclude the valve surgery patients from the primary analysis was the right one at the time.

"Based on the data we had from the Phase II with only 30 patients in the group, I don't think we were in a position to judge whether it had a discernible effect in that population," he said.

Although Schmidt acknowledged that approval of the drug based on PRIMO-CABG data is "far from assured," a biologics license application could be filed as early as 2004, and the revenue potential in the U.S. is between $300 million and $500 million. Alexion's clinical advisers believe the company will "receive a green light to at least file for approval," Schmidt wrote in a research note.

Bell was circumspect, but said the worst-case scenario would be that the FDA decides "the data are in no way supportive, in which case you ought to stop. That's a low likelihood."

Pexelizumab is partnered with Procter &Gamble Pharmaceuticals, an affiliate of Procter &Gamble Co., of Cincinnati. Alexion shares equally in development and commercialization, handling about half of sales efforts and taking half the profits. (See BioWorld Today, Dec. 13, 2001.)

Other secondary endpoints in the Phase III trial include psychomotor function, the effect of pexelizumab on death alone and the drug's effect on perioperative (that is, from the time of hospitalization for surgery to the time of discharge) Q-wave and non-Q-wave myocardial infarction. Details on those endpoints are expected at the AHA meeting to be held in Orlando, Fla.

Meanwhile, the company offered only an outline of the Phase III data, with an opinion of what they might mean. "We believe we have hit a solid triple, and we're now focusing on the best strategy to drive the drug home," Bell said during a conference call, pointing to a significant unmet medical need.

"There are no approved drugs that we are familiar with that limit death or heart attack in this patient population or have been shown to do so," he said.

Behind pexelizumab in the Alexion pipeline is eculizumab, formerly known as 5G1.1, a fully humanized whole antibody (as opposed to single chain), which in Phase I/II and Phase II trials has shown proof- of-principle efficacy in rheumatoid arthritis, lupus/membranous nephritis and dermatomyositis.

Alexion has a manufacturing deal with Lonza Biologics Ltd., of Basel, Switzerland, for eculizumab, which Schmidt said has yielded the most promising data in a Phase I/II study in paroxysmal nocturnal hemoglobinuria, which is associated with a high risk of thrombotic events.

"We would expect over the next several months we would be able to announce our plans [regarding eculizumab]," Bell said.

Subject: Cardiovascular agents -- Product development;Company securities;Pharmaceutical industry -- Product development;Pharmaceutical industry -- Securities;Product development;Securities

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Identifier (keyword): Trade, ALXN

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Kidney Disease; Enrollment complete for phase II membranous nephritis clinical trial with 5G1.1

Publication info: Immunotherapy Weekly (Apr 10, 2002): 18.
ProQuest document link

Abstract (summary): "Completing enrollment in the first eculizumab phase II trial in patients suffering from membranous nephritis brings us closer to our objective of expanding the profile of eculizumab into a variety of chronic autoimmune opportunities," stated Leonard Bell, MD, president and CEO of Alexion. "Once the 4-month treatment period and all follow-up patient visits are completed, we expect to complete data collection and then lock the database for subsequent analysis of this phase II trial.

Full text: Alexion Pharmaceuticals, Inc., (ALXN) announced that it has completed enrollment in its first phase II trial in patients suffering from a severe form of kidney disease known as membranous nephritis, with its anti-inflammatory C5 inhibitor antibody, 5G1.1, now called eculizumab.

Separately, Alexion announced that it has received orphan drug designation from the U.S. Food and Drug Administration for the use of eculizumab, if approved, in patients with membranous nephritis.

This phase II multicenter, double-blind, randomized, placebo- controlled study examines the safety and clinical efficacy of repeated dosing of eculizumab during a 4-month treatment period. Approximately 115 membranous nephritis patients were enrolled in the United States. Alexion also announced that, as a part of its comprehensive development plan for eculizumab, it has already commenced dosing in a follow-on open label extension study of eculizumab in these patients, designed to examine long-term safety and tolerability.

"Completing enrollment in the first eculizumab phase II trial in patients suffering from membranous nephritis brings us closer to our objective of expanding the profile of eculizumab into a variety of chronic autoimmune opportunities," stated Leonard Bell, MD, president and CEO of Alexion. "Once the 4-month treatment period and all follow-up patient visits are completed, we expect to complete data collection and then lock the database for subsequent analysis of this phase II trial. Additionally, we are encouraged by the orphan drug designation as it may provide an innovative therapy for this small, but underserved, patient population, while potentially providing us with development and commercial advantages."

Membranous nephritis is a form of kidney disease that is believed to be caused by a chronic autoimmune disorder.

This article was prepared by Immunotherapy Weekly editors from staff and other reports.

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Kidney Disease; Enrollment complete for phase II membranous nephritis clinical trial with 5G1.1

Publication info: Drug Week (Apr 12, 2002): 14.
ProQuest document link

Abstract (summary): "Completing enrollment in the first eculizumab phase II trial in patients suffering from membranous nephritis brings us closer to our objective of expanding the profile of eculizumab into a variety of chronic autoimmune opportunities," stated Leonard Bell, MD, president and CEO of Alexion. "Once the 4-month treatment period and all follow-up patient visits are completed, we expect to complete data collection and then lock the database for subsequent analysis of this phase II trial.

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Alexion Pharmaceuticals Reports Second Quarter and First Half Results

Publication info: PR Newswire [New York] 08 Mar 2002: 1.
ProQuest document link

Abstract (summary): "Alexion achieved several very significant milestones in the second quarter, including the initiation of the pexelizumab pivotal Phase III PRIMO- CABG trial, completion of patient enrollment in the first of our two acute myocardial infarction Phase II trials with pexelizumab, and the successful restructuring of our collaboration agreement with P&G. We are excited that the revised collaboration with P&G allows Alexion to share in decision-making and development and commercial responsibilities for this important product and provides that we and P&G will share the gross margin on sales in the U.S., on an approximately equal basis," said David Keiser, Executive VP and Chief Operating Officer of Alexion. "Our pipeline has made tremendous progress, also in other areas during the quarter, most noteworthy, being the initiation of a Phase IIb trial of eculizumab, formerly 5G1.1, in rheumatoid arthritis patients."

Alexion is engaged in the discovery and development of therapeutic products aimed at treating patients with a wide array of severe disease states, including cardiovascular and autoimmune disorders, inflammation and cancer. Alexion's two lead product candidates, pexelizumab and eculizumab, are currently in eight clinical development programs. Alexion is developing pexelizumab, an antibody fragment, in collaboration with Procter &Gamble Pharmaceuticals. Together the firms have initiated a Phase III clinical study with pexelizumab in cardiopulmonary bypass patients, and are currently conducting two large Phase II studies with pexelizumab in acute myocardial infarction patients. Alexion's other lead product candidate, eculizumab, is being studied in a Phase IIb trial for the treatment of rheumatoid arthritis. Eculizumab is also in Phase II trials for the treatment of membranous nephritis and for lupus nephritis, and in earlier stage clinical trials for the treatment of dermatomyositis and pemphigoid. Additionally, through its wholly owned subsidiary, Alexion Antibody Technologies, Inc., Alexion is engaged in discovering and developing a portfolio of additional antibody therapeutics targeting severe unmet medical needs. This press release and further information about Alexion Pharmaceuticals, Inc. can be found on the World Wide Web at: http:// www.alexionpharm.com.

Full text: - Quarter Highlighted by Successful Revision of Pexelizumab
Collaboration With Procter &Gamble Pharmaceuticals and Commencement
Of Phase III Pivotal PRIMO-CABG Trial -

CHESHIRE, Conn., March 8 /PRNewswire-FirstCall/ -- Alexion Pharmaceuticals, Inc. (Nasdaq: ALXN) today announced financial results for its second fiscal quarter and half year ended January 31, 2002.

In its second quarter ended January 31, 2002, Alexion received revenues amounting to $3.38 million compared to $3.17 million received in the same period ended January 31, 2001. Revenues were slightly higher than in the same period last year primarily due to receipt of a $2 million milestone payment from our pexelizumab development partner, Procter &Gamble Pharmaceuticals (P&G) for the initiation of our pivotal Phase III clinical trial in CABG patients, called "PRIMO-CABG."

The Company's research and development expenses for the three month period were $15.04 million compared to $9.40 million for the same period last year. The $5.64 million increase resulted principally from greater manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab (formerly 5G1.1), and our incurred ongoing pexelizumab Phase III CABG clinical trial costs. As part of the revised collaboration agreement with P&G signed in December 2001, it was agreed that the Company would bear the first 50% of the projected clinical trial costs for the on-going Phase III PRIMO-CABG study and P&G would bear the second half. As such, in the quarter, we recognized a one-time $3.10 million expense for unbilled reimbursable contract costs associated with manufacturing of pexelizumab for Phase III trials which would have been paid by P&G under the original agreement.

General and administrative expenses were $1.84 million for the quarter compared to $1.97 million for the same period last year, mostly as a result of decreased recruiting costs. Accordingly, total operating expenses increased to $16.88 million for the quarter compared to $11.37 million in the three month period ended January 31, 2001, excluding the non-cash amortization of goodwill. The Company posted interest income for the quarter of $3.92 million compared to $6.54 million for the same period last year. The decrease was primarily due to reduced market interest rates. Interest expense, primarily on the Company's $120 million convertible subordinated notes, was $1.93 million for the quarter versus $1.95 million for the same period last year. A state income tax credit was recognized for the three months ended January 31, 2002 representing a state tax benefit of approximately $700,000.

As a result of the above factors, the Company incurred a net loss for the quarter of $10.81 million, or $0.60 per share versus a net loss of $3.61 million or $0.20 per common share for the same three month period in 2001. Taking into account the non-cash amortization of goodwill related to the Company's acquisition of Prolifaron in September 2000, the Company's total net loss amounted to $4.49 million or $0.25 per common share for the quarter ended January 31, 2001.

For the six months ended January 31, 2002, Alexion's revenues were $5.24 million compared to $6.57 million for the same period last year. The $1.33 million decrease in contract research revenues occurred, despite receipt of the $2 million milestone payment, primarily because clinical trial and clinical manufacturing payments from P&G were lower resulting from last year's completion of the Phase IIb pexelizumab CABG study.

Research and development expenditures were $24.71 million for the first half of this fiscal year compared to $20.32 million for the six months ended January 31, 2001. The increase was, again, attributable to higher clinical manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab, and incurred clinical trial costs for the Company's ongoing pexelizumab Phase III CABG trial.

General and administrative expenses increased slightly to $3.44 million for the six months from $3.35 million for the same period last year mostly as a result of higher payroll costs from added personnel. Accordingly, total operating expenses increased to $28.15 million for the half year compared to $23.67 million, without taking into account last year's acquisition related non-cash charges, in the six month period ended January 31, 2001.

The Company posted interest income for the past six months of $7.46 million compared to $9.34 million for the same period last year. The decrease was primarily due to reduced market interest rates. Interest expense was $3.85 million for the quarter versus $3.95 million for the same period last year. A state income tax credit was recognized in the second quarter ended January 31, 2002 representing a state tax benefit of approximately $700,000.

Net loss for the six months increased to $18.60 million or $1.03 per share from a net loss of $11.70 million or $0.70 per share recorded for the same six month period last year, exclusive of the impact of non-cash charges. Last year's non-cash charges related to Alexion's acquisition of Prolifaron in September 2000 in which the Company recognized a $21.0 million one-time first quarter charge allocated to in-process research and development projects and $1.23 million charge for amortization of goodwill. In addition, the Company recorded a $9.12 million non-cash charge for the cumulative effect of adoption of SAB 101. Taking into account these non-cash charges, the Company's total net loss including operating expenses amounted to $43.05 million or $2.58 per common share for the six months ended January 31, 2001.

As of January 31, 2002 Alexion had approximately $341 million in cash, cash equivalents and marketable securities.

"Alexion achieved several very significant milestones in the second quarter, including the initiation of the pexelizumab pivotal Phase III PRIMO- CABG trial, completion of patient enrollment in the first of our two acute myocardial infarction Phase II trials with pexelizumab, and the successful restructuring of our collaboration agreement with P&G. We are excited that the revised collaboration with P&G allows Alexion to share in decision-making and development and commercial responsibilities for this important product and provides that

we and P&G will share the gross margin on sales in the U.S., on an approximately equal basis," said David Keiser, Executive VP and Chief Operating Officer of Alexion. "Our pipeline has made tremendous progress, also in other areas during the quarter, most noteworthy, being the initiation of a Phase IIb trial of eculizumab, formerly 5G1.1, in rheumatoid arthritis patients."

As a result of the revised collaboration with P&G, of which terms include an approximate 50% share of gross margin on U.S. sales and a royalty on sales in the rest of the world, Alexion will incur approximately 50% of all Phase III clinical trial, product development, manufacturing, and commercialization costs necessary for the potential approval and marketing of pexelizumab in the U.S. Additionally, as part of the agreement, Alexion will generally forgo further research and development support payments from P&G. As a result, Alexion anticipates its contract research revenues will not exceed $7.0 million for the current fiscal year, and operating expenses will increase significantly going forward, reaching $78 to $82 million by the end of our fiscal year in July 31, 2002. Net loss for the year ending July 31, 2002 is expected to be in the $65 to $70 million range.

Alexion is engaged in the discovery and development of therapeutic products aimed at treating patients with a wide array of severe disease states, including cardiovascular and autoimmune disorders, inflammation and cancer. Alexion's two lead product candidates, pexelizumab and eculizumab, are currently in eight clinical development programs. Alexion is developing pexelizumab, an antibody fragment, in collaboration with Procter &Gamble Pharmaceuticals. Together the firms have initiated a Phase III clinical study with pexelizumab in cardiopulmonary bypass patients, and are currently conducting two large Phase II studies with pexelizumab in acute myocardial infarction patients. Alexion's other lead product candidate, eculizumab, is being studied in a Phase IIb trial for the treatment of rheumatoid arthritis. Eculizumab is also in Phase II trials for the treatment of membranous nephritis and for lupus nephritis, and in earlier stage clinical trials for the treatment of dermatomyositis and pemphigoid. Additionally, through its wholly owned subsidiary, Alexion Antibody Technologies, Inc., Alexion is engaged in discovering and developing a portfolio of additional antibody therapeutics targeting severe unmet medical needs. This press release and further information about Alexion Pharmaceuticals, Inc. can be found on the World Wide Web at: http:// www.alexionpharm.com.

This news release contains forward-looking statements. Such statements are subject to certain factors which may cause Alexion's plans to differ or results to vary from those expected, including the results of pre-clinical or clinical studies (including termination or delay in clinical programs or inability to move forward to the next Phase of clinical development), the need for additional research and testing, delays in developing or arranging satisfactory manufacturing capability, inability to access capital and funding on a timely basis and on favorable terms, delays in development of or adverse changes in status of commercial relationships, the possibility that favorable results of earlier clinical trials are not predictive of safety and efficacy results in larger clinical trials, dependence on Procter &Gamble Pharmaceuticals for performance of development and commercial matters related to pexelizumab, the risk that third parties won't agree to license us on reasonable terms their intellectual property necessary for us to develop and commercialize our products, and a variety of other risks set forth from time to time in Alexion's filings with the Securities and Exchange Commission, including but not limited to the risks discussed in Alexion's Annual Report on Form 10-K for the year ended July 31, 2001. Except in special circumstances in which a duty to update arises under law when prior disclosure becomes materially misleading in light of subsequent events, Alexion does not intend to update any of these forward- looking statements to reflect events or circumstances after the date hereof or to reflect the occurrence of unanticipated events.

ALEXION PHARMACEUTICALS, INC.

Selected Financial Data

Statements of Operations

(dollars in thousands except per share amounts)

Quarter ended Quarter ended
January 31, January 31,
2002 2001 2001
Pro forma -a)
REVENUES $3,380 $3,174

$3,174

OPERATING EXPENSES:

Research and Development 15,043 9,399
9,399
General and Administrative 1,836 1,971

1,971

In-Process Research

and Development (IPRD) -- --
--
Amortization of Goodwill -- --
878
Total Operating Expenses 16,879 11,370
12,248
OPERATING INCOME (LOSS) (13,499) (8,196)
(9,074)
Interest Income 3,918 6,536
6,536
Interest Expense (1,929) (1,948)
(1,948)
State Tax Benefit 700 --

--
NET INCOME (LOSS) $(10,810) $(3,608) -a)

$(4,486)
BASIC AND DILUTED NET INCOME

(LOSS) PER COMMON SHARE $(0.60) $(0.20) -a)

$(0.25)
SHARES USED IN COMPUTING BASIC
AND DILUTED NET INCOME (LOSS)

PER COMMON SHARE 18,119 17,999
17,999
-a) excludes non-cash IPRD and Amortization of Goodwill
ALEXION PHARMACEUTICALS, INC.

Selected Financial Data
Statements of Operations
(dollars in thousands except per share amounts)
Six Months ended Six Months ended
January 31, January 31,

2002 2001 2001
Pro forma -a)
REVENUES $5,240 $6,573

$6,573
OPERATING EXPENSES:

Research and Development 24,714 20,322
20,322
General and Administrative 3,435 3,349

3,349
In-Process Research

and Development (IPRD) -- --

21,000
Amortization of Goodwill -- --
1,227
Total Operating Expenses 28,149 23,671
45,898
OPERATING INCOME (LOSS) (22,909) (17,098)
(39,325)
Interest Income 7,456 9,344
9,344
Interest Expense (3,846) (3,946)
(3,946)
State Tax Benefit 700 --

--

Cumulative Effect of

Adoption of SAB 101 -- --
(9,118)
NET INCOME (LOSS) $(18,599) $(11,700) -a)

$(43,045)

BASIC AND DILUTED NET INCOME

(LOSS) PER COMMON SHARE $(1.03) $(0.70) -a)

$(2.58)

SHARES USED IN COMPUTING BASIC AND

DILUTED NET INCOME (LOSS) PER

COMMON SHARE 18,114 16,661

16,661

- a) excludes non-cash IPRD, Amortization of Goodwill, and Cumulative

Effect of Adoption of SAB 101

Balance Sheet Data

(dollars in thousands)
January 31, January 31, July 31,

2002 2001 2001
(unaudited) (unaudited) (audited)
Cash, cash equivalents and
marketable securities $340,939 $369,904
$355,274
Total Assets $383,515 $414,515
$400,259
Net Stockholders' Equity $242,368 $282,214

$260,408
Contacts: Alexion Pharmaceuticals, Inc.
David Keiser
Executive Vice President &COO
(203) 272-2596
Noonan/Russo Communications, Inc. Rx Communications

Ernie Knewitz (Media) Rhonda Chiger (Investor)
(212) 696-4455 Ext. 204 917-322-2569
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http://tbutton.prnewswire.com/prn/11690X78322878 SOURCE Alexion Pharmaceuticals, Inc.

References

Message No: Industry: MEDICAL/PHARMACEUTICALS;

Company / organization: Company / organization Name: Procter &Gamble Pharmaceuticals; SIC: 2830;
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Alexion Pharmaceuticals Reports Second Quarter and First Half Results; - Quarter Highlighted by Successful Revision of Pexelizumab Collaboration With Procter &Gamble Pharmaceuticals and Commencement Of Phase III Pivotal PRIMO-CABG Trial -

Publication info: PR Newswire 08 Mar 2002: NA.

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Abstract (English): CHESHIRE, Conn. -- Alexion Pharmaceuticals, Inc. today announced financial results for its second fiscal quarter and half year ended January 31, 2002.

In its second quarter ended January 31, 2002, Alexion received revenues amounting to $3.38 million compared to $3.17 million received in the same period ended January 31, 2001. Revenues were slightly higher than in the same period last year primarily due to receipt of a $2 million milestone payment from our pexelizumab development partner, Procter &Gamble Pharmaceuticals (P&G) for the initiation of our pivotal Phase III clinical trial in CABG patients, called "PRIMO-CABG."

The Company's research and development expenses for the three month period were $15.04 million compared to $9.40 million for the same period last year.

Full text: CHESHIRE, Conn. -- Alexion Pharmaceuticals, Inc. today announced financial results for its second fiscal quarter and half year ended January 31, 2002.

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The Company's research and development expenses for the three month period were $15.04 million compared to $9.40 million for the same period last year. The $5.64 million increase resulted principally from greater manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab (formerly 5G1.1), and our incurred ongoing pexelizumab Phase III CABG clinical trial costs. As part of the revised collaboration agreement with P&G signed in December 2001, it was agreed that the Company would bear the first 50% of the projected clinical trial costs for the on-going Phase III PRIMO-CABG study and P&G would bear the second half. As such, in the quarter, we recognized a one-time $3.10 million expense for unbilled reimbursable contract costs associated with manufacturing of pexelizumab for Phase III trials which would have been paid by P&G under the original agreement.

General and administrative expenses were $1.84 million for the quarter compared to $1.97 million for the same period last year, mostly as a result of decreased recruiting costs. Accordingly, total operating expenses increased to $16.88 million for the quarter compared to $11.37 million in the three month period ended January 31, 2001, excluding the non-cash amortization of goodwill. The Company posted interest income for the quarter of $3.92 million compared to $6.54 million for the same period last year. The decrease was primarily due to reduced market interest rates. Interest expense, primarily on the Company's $120 million convertible subordinated notes, was $1.93 million for the quarter versus $1.95 million for the same period last year. A state income tax credit was recognized for the three months ended January 31, 2002 representing a state tax benefit of approximately $700,000.

As a result of the above factors, the Company incurred a net loss for the quarter of $10.81 million, or $0.60 per share versus a net loss of $3.61 million or $0.20 per common share for the same three month period in 2001. Taking into account the non-cash amortization of goodwill related to the Company's acquisition of Prolifaron in September 2000, the Company's total net loss amounted to $4.49 million or $0.25 per common share for the quarter ended January 31, 2001.

For the six months ended January 31, 2002, Alexion's revenues were $5.24 million compared to $6.57 million for the same period last year. The $1.33 million decrease in contract research revenues occurred, despite receipt of the $2 million milestone payment, primarily because clinical trial and clinical manufacturing payments from P&G were lower resulting from last year's completion of the Phase IIb pexelizumab CABG study.
Research and development expenditures were $24.71 million for the first half of this fiscal year compared to $20.32 million for the six months ended January 31, 2001. The increase was, again, attributable to higher clinical manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab, and incurred clinical trial costs for the Company's ongoing pexelizumab Phase III CABG trial.
General and administrative expenses increased slightly to $3.44 million for the six months from $3.35 million for the same period last year mostly as a result of higher payroll costs from added personnel. Accordingly, total operating expenses increased to $28.15 million for the half year compared to $23.67 million, without taking into account last year's acquisition related non-cash charges, in the six month period ended January 31, 2001.
The Company posted interest income for the past six months of $7.46 million compared to $9.34 million for the same period last year. The decrease was primarily due to reduced market interest rates. Interest expense was $3.85 million for the quarter versus $3.95 million for the same period last year. A state income tax credit was recognized in the second quarter ended January 31, 2002 representing a state tax benefit of approximately $700,000.
Net loss for the six months increased to $18.60 million or $1.03 per share from a net loss of $11.70 million or $0.70 per share recorded for the same six month period last year, exclusive of the impact of non-cash charges. Last year's non-cash charges related to Alexion's acquisition of Prolifaron in September 2000 in which the Company recognized a $21.0 million one-time first quarter charge allocated to in-process research and development projects and $1.23 million charge for amortization of goodwill. In addition, the Company recorded a $9.12 million non-cash charge for the cumulative effect of adoption of SAB 101. Taking into account these non-cash charges, the Company's total net loss including operating expenses amounted to $43.05 million or $2.58 per common share for the six months ended January 31, 2001.
As of January 31, 2002 Alexion had approximately $341 million in cash, cash equivalents and marketable securities.
"Alexion achieved several very significant milestones in the second quarter, including the initiation of the pexelizumab pivotal Phase III PRIMO- CABG trial, completion of patient enrollment in the first of our two acute myocardial infarction Phase II trials with pexelizumab, and the successful restructuring of our collaboration agreement with P&G. We are excited that the revised collaboration with P&G allows Alexion to share in decision-making and development and commercial responsibilities for this important product and provides that we and P&G will share the gross margin on sales in the U.S., on an approximately equal basis," said David Keiser, Executive VP and Chief Operating Officer of Alexion. "Our pipeline has made tremendous progress, also in other areas during the quarter, most noteworthy, being the initiation of a Phase IIb trial of eculizumab, formerly 5G1.1, in rheumatoid arthritis patients."
As a result of the revised collaboration with P&G, of which terms include an approximate 50% share of gross margin on U.S. sales and a royalty on sales in the rest of the world, Alexion will incur approximately 50% of all Phase III clinical trial, product development, manufacturing, and commercialization costs necessary for the potential approval and marketing of pexelizumab in the U.S. Additionally, as part of the agreement, Alexion will generally forgo further research and development support payments from P&G. As a result, Alexion anticipates its contract research revenues will not exceed $7.0 million for the current fiscal year, and operating expenses will increase significantly going forward, reaching $78 to $82 million by the end of our fiscal year in July 31, 2002. Net loss for the year ending July 31, 2002 is expected to be in the $65 to $70 million range.
Alexion is engaged in the discovery and development of therapeutic products aimed at treating patients with a wide array of severe disease states, including cardiovascular and autoimmune disorders, inflammation and

cancer. Alexion's two lead product candidates, pexelizumab and eculizumab, are currently in eight clinical development programs. Alexion is developing pexelizumab, an antibody fragment, in collaboration with Procter &Gamble Pharmaceuticals. Together the firms have initiated a Phase III clinical study with pexelizumab in cardiopulmonary bypass patients, and are currently conducting two large Phase II studies with pexelizumab in acute myocardial infarction patients. Alexion's other lead product candidate, eculizumab, is being studied in a Phase IIb trial for the treatment of rheumatoid arthritis. Eculizumab is also in Phase II trials for the treatment of membranous nephritis and for lupus nephritis, and in earlier stage clinical trials for the treatment of dermatomyositis and pemphigoid. Additionally, through its wholly owned subsidiary, Alexion Antibody Technologies, Inc., Alexion is engaged in discovering and developing a portfolio of additional antibody therapeutics targeting severe unmet medical needs. This press release and further information about Alexion Pharmaceuticals, Inc. can be found on the World Wide Web at: http://www.alexionpharm.com/.

This news release contains forward-looking statements. Such statements are subject to certain factors which may cause Alexion's plans to differ or results to vary from those expected, including the results of pre-clinical or clinical studies (including termination or delay in clinical programs or inability to move forward to the next Phase of clinical development), the need for additional research and testing, delays in developing or arranging satisfactory manufacturing capability, inability to access capital and funding on a timely basis and on favorable terms, delays in development of or adverse changes in status of commercial relationships, the possibility that favorable results of earlier clinical trials are not predictive of safety and efficacy results in larger clinical trials, dependence on Procter &Gamble Pharmaceuticals for performance of development and commercial matters related to pexelizumab, the risk that third parties won't agree to license us on reasonable terms their intellectual property necessary for us to develop and commercialize our products, and a variety of other risks set forth from time to time in Alexion's filings with the Securities and Exchange Commission, including but not limited to the risks discussed in Alexion's Annual Report on Form 10-K for the year ended July 31, 2001. Except in special circumstances in which a duty to update arises under law when prior disclosure becomes materially misleading in light of subsequent events, Alexion does not intend to update any of these forward-looking statements to reflect events or circumstances after the date hereof or to reflect the occurrence of unanticipated events.

ALEXION PHARMACEUTICALS, INC.

Selected Financial Data

Statements of Operations

(dollars in thousands except per share amounts)

	Quarter ended January 31, 2002	Quarter ended January 31, 2001	2001 Pro forma -a)
REVENUES	$3,380	$3,174	$3,174
OPERATING EXPENSES:			
Research and Development	15,043	9,399	9,399
General and Administrative	1,836	1,971	1,971
In-Process Research and Development (IPRD)	--	--	--
Amortization of Goodwill	--	--	878
Total Operating Expenses	16,879	11,370	12,248
OPERATING INCOME (LOSS)	(13,499)	(8,196)	(9,074)
Interest Income	3,918	6,536	6,536
Interest Expense	(1,929)	(1,948)	(1,948)
State Tax Benefit	700	--	--

NET INCOME (LOSS) $(10,810) $(3,608) -a) $(4,486)
BASIC AND DILUTED NET INCOME
(LOSS) PER COMMON SHARE $(0.60) $(0.20) -a) $(0.25)
SHARES USED IN COMPUTING BASIC
AND DILUTED NET INCOME (LOSS)
PER COMMON SHARE 18,119 17,999 17,999
-a) excludes non-cash IPRD and Amortization of Goodwill
ALEXION PHARMACEUTICALS, INC.
Selected Financial Data
Statements of Operations
(dollars in thousands except per share amounts)
Six Months ended Six Months ended
January 31, January 31,
2002 2001 2001
Pro forma -a)
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Cumulative Effect of
Adoption of SAB 101 -- -- (9,118)
NET INCOME (LOSS) $(18,599) $(11,700) -a) $(43,045)
BASIC AND DILUTED NET INCOME
(LOSS) PER COMMON SHARE $(1.03) $(0.70) -a) $(2.58)
SHARES USED IN COMPUTING BASIC AND
DILUTED NET INCOME (LOSS) PER
COMMON SHARE 18,114 16,661 16,661
- a) excludes non-cash IPRD, Amortization of Goodwill, and Cumulative
Effect of Adoption of SAB 101
Balance Sheet Data
(dollars in thousands)
January 31, January 31, July 31,
2002 2001 2001
(unaudited) (unaudited) (audited)
Cash, cash equivalents and
marketable securities $340,939 $369,904 $355,274
Total Assets $383,515 $414,515 $400,259
Net Stockholders' Equity $242,368 $282,214 $260,408

Contacts: Alexion Pharmaceuticals, Inc.
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Noonan/Russo Communications, Inc. Rx Communications
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(212) 696-4455 Ext. 204 917-322-2569
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http://tbutton.prnewswire.com/prn/11690X78322878

Contact: David Keiser, Executive Vice President &COO of Alexion Pharmaceuticals, Inc., +1-203-272-2596; Media - Ernie Knewitz of Noonan-Russo Communications, Inc., +1-212-696-4455, ext. 204; Investors - Rhonda Chiger of Rx Communications, +1-917-322-2569, both for Alexion Pharmaceuticals, Inc.
Website: http://www.alexionpharm.com/

Subject: Drugs;Pharmaceutical industry

Company / organization: Alexion Pharmaceuticals Inc.; Procter &Gamble Pharmaceuticals

Identifier (keyword): Trade, ALXN

NAICS classification: 325412: Pharmaceutical Preparation Manufacturing

Product classification: 2834000: Pharmaceutical Preparations

SIC classification: 2834: Pharmaceutical preparations

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Alexion Pharmaceuticals, Inc.; Company reports second quarter, first- half results

Publication info: Biotech Week (Apr 10, 2002): 9.
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Abstract (summary): "Alexion achieved several very significant milestones in the second quarter, including the initiation of the pexelizumab pivotal phase III PRIMO-CABG trial, completion of patient enrollment in the first of our two acute myocardial infarction phase II trials with pexelizumab, and the successful restructuring of our collaboration agreement with P &G.

Full text: Alexion Pharmaceuticals, Inc., (ALXN) announced financial results for its second fiscal quarter and half year ended January 31, 2002.

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The company's research and development expenses for the 3-month period were $15.04 million compared with $9.40 million for the same period last year. The $5.64 million increase resulted principally from greater manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab (formerly 5G1.1), and our incurred ongoing pexelizumab phase III CABG clinical trial costs. As part of the revised collaboration agreement with P &G signed in December 2001, it was agreed that the company would bear the first 50% of the projected clinical trial costs for the on- going phase III PRIMO-CABG study and P &G would bear the second half. As such, in the quarter, we recognized a one-time $3.10 million expense for unbilled reimbursable contract costs associated with manufacturing of pexelizumab for phase III trials which would have been paid by P &G under the original agreement.

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As a result of the above factors, the company incurred a net loss for the quarter of $10.81 million, or $0.60 per share versus a net loss of $3.61 million or $0.20 per common share for the same 3- month period in 2001. Taking into account the noncash amortization of goodwill related to the company's acquisition of Prolifaron in September 2000, the company's total net loss amounted to $4.49 million or $0.25 per common share for the quarter ended January 31, 2001.

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Research and development expenditures were $24.71 million for the first half of this fiscal year compared with $20.32 million for the 6 months ended January 31, 2001. The increase was, again, attributable to higher clinical manufacturing costs associated with our lead C5 inhibitor candidates, pexelizumab and eculizumab, and incurred clinical trial costs for the company's ongoing pexelizumab phase III CABG trial.

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"Alexion achieved several very significant milestones in the second quarter, including the initiation of the pexelizumab pivotal phase III PRIMO-CABG trial, completion of patient enrollment in the first of our two acute

myocardial infarction phase II trials with pexelizumab, and the successful restructuring of our collaboration agreement with P &G. We are excited that the revised collaboration with P &G allows Alexion to share in decision-making and development and commercial responsibilities for this important product and provides that we and P &G will share the gross margin on sales in the U.S., on an approximately equal basis," said David Keiser, executive VP and chief operating officer of Alexion. "Our pipeline has made tremendous progress, also in other areas during the quarter, most noteworthy, being the initiation of a phase IIb trial of eculizumab, formerly 5G1.1, in rheumatoid arthritis patients."
This article was prepared by Biotech Week editors from staff and other reports.

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THERAPEUTIC ANTIBODIES FOR HUMAN DISEASES AT THE DAWN OF THE TWENTY-FIRST CENTURY

Ole Henrik Brekke and Inger Sandlie‡*

Antibodies are highly specific, naturally evolved molecules that recognize and eliminate pathogenic and disease antigens. The past 30 years of antibody research have hinted at the promise of new versatile therapeutic agents to fight cancer, autoimmune diseases and infection. Technology development and the testing of new generations of antibody reagents have altered our view of how they might be used for prophylactic and therapeutic purposes. The therapeutic antibodies of today are genetically engineered molecules that are designed to ensure high specificity and functionality. Some antibodies are loaded with toxic modules, whereas others are designed to function naturally, depending on the therapeutic application. In this review, we discuss various aspects of antibodies that are relevant to their use as as therapeutic agents.

IMMUNOGLOBULIN DOMAIN
Compactly folded globular units of approximately 110 amino acids that comprise immunoglobulin heavy and light chains.

CYTOKINES
A class of small proteins released by one cell that affects the physiology of other cells locally and systemically in a particular fashion through binding to a specific receptor.

**Affitech AS, Gaustadalleen 21, N-0349 Oslo, Norway. ‡Department of Biology, University of Oslo, N-0349, Norway. Correspondence to O.H.B. e-mail: o.h.brekke@affitech.com*
doi:10.1038/nrd984

Antibody structure

The typical antibody — or immunoglobulin (Ig) — consists of two antigen-binding fragments (Fabs), which are linked via a flexible region (the hinge) to a constant (Fc) region (FIG. 1). This structure comprises two pairs of polypeptide chains, each pair containing a heavy and a light chain of different sizes. Both heavy and light chains are folded into IMMUNOGLOBULIN DOMAINS. The 'variable domains' in the amino-terminal part of the molecule are the domains that recognize and bind antigens; the rest of the molecule is composed of 'constant domains' that only vary between Ig classes (BOX 1). The Fc portion of the Ig serves to bind various effector molecules of the immune system, as well as molecules that determine the biodistribution of the antibody.

Mechanisms of *in vivo* action

In antibody-based therapies, the goal is to eliminate or neutralize the pathogenic infection or the disease target, for example, bacterial, viral or tumour targets. Therapeutic antibodies can function by three principal modes of action: by blocking the action of specific molecules, by targeting specific cells or by functioning as signalling molecules. The blocking activity of therapeutic antibodies is achieved by preventing growth factors, CYTOKINES or other soluble mediators reaching their target receptors, which can be accomplished either by the antibody binding to the factor itself or to its receptor. Targeting involves directing antibodies towards specific populations of cells and is a versatile approach; antibodies can be engineered to carry effector moieties, such as enzymes, toxins, radionuclides, cytokines or even DNA molecules, to the target cells, where the attached moiety can then exert its effect (for example, toxins or radionuclides can eliminate target cancer cells). The natural EFFECTOR FUNCTIONS of antibodies are associated with binding to Fc receptors or binding to complement proteins and inducing COMPLEMENT-DEPENDENT CYTOTOXICITY (CDC). Targeting antibodies can retain such effector functions intact or they can be abolished during the design of the antibody, depending on the therapeutic strategy. The signalling effect of antibodies is predicated on either inducing crosslinking of receptors that are, in turn, connected to mediators of cell division or PROGRAMMED CELL DEATH, or directing them towards specific receptors to act as agonists for the

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Page 375

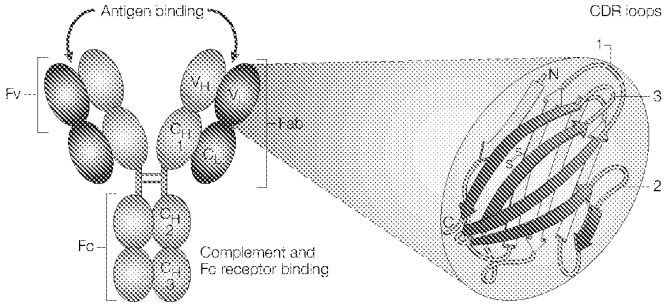

Figure 1 | **The modular structure of immunoglobulins.** This figure shows a single immunoglobulin (Ig) molecule. All immunoglobulin monomers are composed of two identical light (L) chains and two identical heavy (H) chains. Light chains are composed of one constant domain (C_L) and one variable domain (V_L), whereas heavy chains are composed of three constant domains (C_H1, C_H2 and C_H3) and one variable domain (V_H). The heavy chains are covalently linked in the hinge region and the light chains are covalently linked to the heavy chain. The variable domains of both the heavy and light chains compose the antigen-binding part of the molecule, termed Fv. Within the variable domains there are three loops designated complementarity-determining regions (CDRs) 1, 2 and 3, which confer the highest diversity and define the specificity of antibody binding. The Fc portion is glycosylated and contains the sites for interaction with effector molecules, such as the C1 complex of the complement system and a variety of Fc receptors including the neonatal Fc receptor (FcRn).

activation of specific cell populations. Another approach is to use antibodies as delivery vehicles for DNA and, more recently, to deliver antigens to certain immune cells that present processed antigenic peptides, or epitopes, to T cells, to activate a specific immune response against that antigen.

EFFECTOR FUNCTIONS
The antigen-elimination processes mediated by immunoglobulins and initiated by the binding of effector molecules to the Fc part of the immunoglobulin. The common effector functions are complement-dependent cytotoxicity (CDC), phagocytosis and antibody-dependent cellular cytotoxicity (ADCC).

COMPLEMENT-DEPENDENT CYTOTOXICITY
Once bound to antigen, both IgM and IgG can trigger a sequence of reactions by which serum proteins called complement factors are cleaved. One of the results is destruction of the target cell through complement-dependent cytotoxicity.

PROGRAMMED CELL DEATH
Programmed cell death infers that cells are determined to die at a specific stage of development or having received a specific signal. The process is known as apoptosis. The cells shrivel and are engulfed by nearby phagocytic cells without eliciting any inflammatory response.

The development of therapeutic antibodies

Target specificity in the treatment and prophylaxis of diseases such as infection, cancer and autoimmune disorders has become more viable through the development of monoclonal antibodies. The mouse HYBRIDOMA technology described by Köhler and Milstein was an important step in the development of antibody technology and paved the way for the emergence of therapeutic monoclonal antibodies[1]. During the 1980s, resources were directed towards the evaluation of the *in vivo* use of mouse monoclonal antibodies in humans, aimed at both imaging and therapy[2]. Mouse monoclonal antibodies were shown to have limited use as therapeutic agents because of a short serum half-life, an inability to trigger human effector functions and the production of human antimouse-antibodies[3] (the HAMA response). In an attempt to reduce the immunogenicity of mouse antibodies, genetic engineering was used to generate chimeric antibodies, that is, antibodies with human constant regions and mouse variable regions[4,5]. However, although chimeric antibodies were perceived as less foreign, and therefore less immunogenic, than mouse monoclonal antibodies, human anti-chimeric antibody responses (HACAs) have nonetheless been observed[6]. Further minimization of the mouse component of antibodies was achieved through CDR (complementarity-determining region) grafting[7] (FIG. 2). In such 'humanized' antibodies, only the CDR loops that are responsible for antigen binding are inserted into the human variable-domain framework. The ability to manipulate antibodies into more human variants finally made antibodies useful for clinical use. With the isolation of genes encoding human variable regions, their successful expression in *Escherichia coli*[8,9] and the introduction of phage-display technology[10], the task of selecting fully human variable domains has been greatly simplified.

Selection from phage-display libraries of human antibody fragments is today the most used and well-established technology for the development of new human antibodies (FIG. 3a). Another approach is to use mice that are transgenic for the human Ig locus[11]. Immunization of such a transgenic mouse results in a human antibody response, from which hybridomas that produce human antibodies can be generated (FIG. 3b).

Today there are approximately 200 antibodies in clinical trials and the US Food and Drug Administration has approved several antibodies against cancer[12,13], transplant rejection[14], rheumatoid arthritis and Crohn's disease[15,16], and antiviral prophylaxis[17] (TABLE 1). So far, 20% of all biopharmaceuticals in clinical trials are monoclonal antibodies (examples are shown in TABLE 2, and see clinical trials web site in online links box), making this the second largest biopharmaceutical product category after vaccines. As the development of potential new therapeutic agents into commercial products takes about 10 years, the FDA-approved antibodies, and some of those in the end-stages of development pipelines, are chimeric or humanized antibodies that were developed with early antibody engineering technologies. The more recently developed reagents, on the other hand, are completely human antibodies that are derived from phage antibody libraries and transgenic mice[17–20].

Antibodies against infectious agents

In the pre-antibiotic era, SERUM THERAPY was widely used as treatment for infectious diseases such as anthrax, smallpox, meningitis and the plague. With the introduction of vaccines and antibiotics, the use of serum therapy declined. However, with the reduction and eradication of certain diseases (for example, smallpox), which has had the result that populations are no longer routinely vaccinated against these infectious agents, and the increasing emergence of antibiotic-resistant bacteria, humans are susceptible to acute outbreaks of disease or to biological terrorism. Antibiotics are obviously ineffective at eliminating viral infections, and the antiviral drugs in use today are often associated with a short serum half-life and resistance often emerges after repeated use[21]. Passive antibody serum therapy is today used merely in replacement therapy for patients with immune disorders, for POST-EXPOSURE PROPHYLAXIS against several viruses (for example, rabies, measles, hepatitis A and B, varicella and respiratory syncitial virus (RSV)) and for toxin neutralization (for example, diphtheria, botulism and tetanus). Passive immunization has substantial advantages over the administration of antimicrobial agents, including low toxicity and high specific

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Box 1 | Immunoglobulin classes

Antibodies belong to either one of five immunoglobulin (Ig) classes: IgA, IgD, IgE, IgG or IgM. Each class has a distinct structure and biological activity. Some of the classes are further divided into subclasses — for example, there are four IgG subclasses and two IgA subclasses. IgM is the first antibody to be produced in an immune response and forms a pentameric complex comprised of Ig monomers. IgA is the main class of antibody in external secretions, where it is found as a dimer that protects the body's mucosal surfaces from infection; it is also found as a monomer in serum. IgD is the main antibody on the surface of B cells. IgE is found bound to cells that secrete histamines after antigen binding. IgG is the main antibody in serum. The IgG class is the most stable and has a serum half-life of 20 days, whereas IgM and IgA persist for only 5–8 days. Both IgM and IgG can mediate complement fixation, whereas only IgG can promote antibody-dependent cellular cytotoxicity (ADCC). IgA, and to a certain degree IgM, can mediate trancytosis to mucosal surfaces, whereas only IgG can be transported across placenta for fetal protection.

activity, as well as an immediate effect compared with vaccines and even antibiotics. Given these issues, biotechnology companies and institutions working within the field of infectious disease protection are certain to direct efforts towards developing highly effective and functional antibody candidates against specific disease targets.

The isolation of protective toxin-neutralizing human monoclonal antibodies was described in 1993 in a study in which several human monoclonal antibodies against tetanus toxin were isolated and a protective effect against tetanus toxin was observed[22]. However, when combinations of these specific monoclonal antibodies were administered, an extra potent (that is, synergistic) effect was observed. Such an effect has also recently been described in the neutralization of botulinum neurotoxin. The anti-botulinum toxin antibodies were derived from different phage-display libraries obtained from humans or immunized mice (J. Marks, presented at Cambridge Healthtech Institute conference on Recombinant Antibodies, Cambridge, Massachusetts, USA, 24–25 April 2002). Recently, Maynard and colleagues described high-affinity antibodies against *Bacillus anthracis*[23]. These antibodies were fragments derived from variable-chain genes of a mouse monoclonal antibody and expressed in *E. coli* as single-chain Fv fragments (scFvs). By administering the antibody fragments to mice before injection of anthrax toxin, protection against the toxin was observed. Protective human antibodies against Shiga-toxin1 were recently isolated by the immunization of transgenic mice[20]. The panel of ten different antibodies of the IgM and IgG1 class that showed specificities to different subunits of the toxin effectively neutralized the toxin.

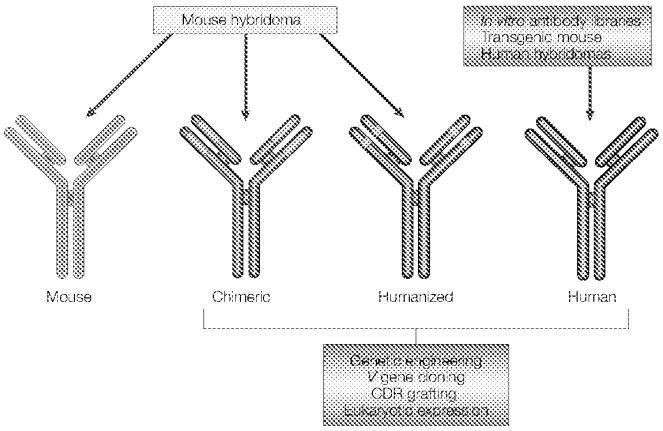

Figure 2 | **Antibody engineering.** Mouse hybridoma technology generates mouse monoclonal antibodies. Genetic engineering has fostered the generation of chimeric, humanized and human antibodies. Cloning of mouse variable genes into human constant-region genes generates chimeric antibodies. Humanized antibodies are generated by the insertion of mouse complementarity-determining regions (CDRs) onto human constant and variable domain frameworks; however, additional changes in the framework regions have, in several cases, been shown to be crucial in maintaining identical antigen specificity[75,76]. Fully human antibodies can be generated by the selection of human antibody fragments from *in vitro* libraries (see BOX 2 and FIG. 3a), by transgenic mice (FIG. 3b) and through selection from human hybridomas.

Compared with toxin neutralization, the use of antibodies in the prevention of viral disease is a more complex prospect. Palivizumab (Synagis; MedImmune Inc.) is a humanized IgG1 monoclonal antibody approved for the prevention of RSV infections in high-risk infants[17,24]. Palivizumab was the first monoclonal antibody approved for an infectious pathogen and is, so far, the only antiviral monoclonal antibody in clinical use. The development of sevirumab (Protovir; Protein Design Labs) — a human anti-cytomegalovirus (CMV) antibody — was, by contrast, halted in Phase III clinical trials as a supplemental treatment for CMV-induced retinitis because of a lack of evidence of efficacy. The elimination of a viral infection requires that a number of events occur, including inhibition of cell infection, mediation of cell killing of infected cells, inhibition of viral replication, inhibition of viral release and inhibition of cell–cell transmission[25,26]. Within the multitude of antibody specificities generated in the typical human polyclonal response, it is likely that one or several effective antibodies against one or more of the particular processes listed will be found. Some of these antibodies bind neutralizing epitopes, whereas others bind non-neutralizing epitopes. The overall outcome might indeed be effective protection due to the combination of the blocking, neutralizing and eliminating effect of human antibodies. Recently, XTL Pharmaceuticals reported on Phase I/II clinical trials with two human monoclonal antibodies against hepatitis B virus[28]. The two monoclonal antibodies were combined with lamivdudine (Epivir-HBV; GlaxoWellcome) — an antiviral drug that inhibits DNA replication — and showed significant reduction in serum viral titre. If monoclonal antibodies are to be used as prophylactics or therapeutics against infectious diseases, it is likely that their efficacy will be increased when a polyclonal passive human serum therapy is mimicked — that is, when a pool of highly specific and high-affinity monoclonal antibodies are administered. There are, however, limitations to this approach, including the production costs associated with manufacturing intact human antibodies.

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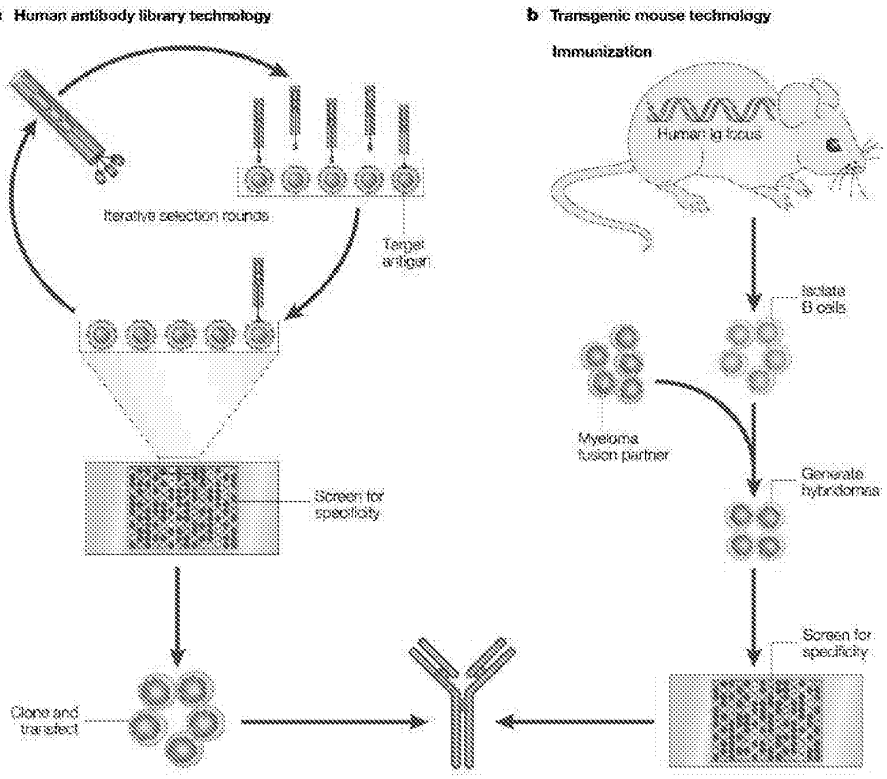

Figure 3 | ***In vitro* and *in vivo* human antibody techniques exemplified by phage display and transgenic mouse technologies. a** | The *in vitro* process is based on panning the library of antibodies against an immobilized target. The non-binding phage antibodies are washed away and the recovered antibodies are amplified by infection in *Escherichia coli*. The selection rounds are subsequently repeated until the desired specificity is obtained. The antibody format for screening is either Fab or single-chain Fv. The expression of antibodies in *E. coli* and recent developments in screening technologies[77] have made it possible to screen tens of thousands of clones for specificity. The antibody fragments themselves can be used as therapeutic agents as discussed in this review, but they can also be converted into intact immunoglobulins by the cloning of the variable genes into plasmids incorporating the constant-region genes of immunoglobulins. The genes are transfected into cell lines and therefore produce fully human immunoglobulins. **b** | The *in vivo* process is based on the immunization of a transgenic mouse. The mouse has been genetically engineered and bred for the expression of human immunoglobulins. The B cells harvested after immunization can be immortalized by fusion with a myeloma cell line, as in traditional hybridoma technology. The hybridomas can then be screened for specific antibodies.

HYBRIDOMA
An antibody-secreting B-cell line that is generated by fusing splenic-derived B cells with a plasmacytoma. A hybridoma produces the same antibody as the parent B cell and divides and grows in culture like the parent cancer cell. The antibody produced is monoclonal.

SERUM THERAPY
The treatment of an infectious disease with the serum from an immunized animal or individual, and which contains antibody.

POST-EXPOSURE PROPHYLAXIS
A treatment that is designed to protect an individual against a disease agent to which the individual has been recently exposed.

A possible solution to this problem is to instead use blocking antibody fragments synthesized in *E. coli*, which could be used to combat, for example, viral infections. A further difficulty in developing such multi-antibody therapeutics might arise from regulatory concerns regarding the administration of multiple monoclonal antibodies. The experience obtained from XTL Pharmaceuticals' use of combined antibodies might be an important step in resolving — or exacerbating — these concerns.

Anti-inflammatory antibodies

Antibodies with high specificity and affinity can be developed to bind specific cytokines or their receptors. In both cases, the purpose is to inhibit the detrimental effect of the cytokine. Cytokines associated with inflammation and autoimmunity include tumour-necrosis factor-α (TNF-α), interleukins and complement proteins. Modulation of immune-responses, such as immune-cell depletion by the targeting of antibodies to cell-surface receptors, for example, CD20 and CD4 on B or T cells, has also been shown as a viable therapeutic strategy in autoimmune diseases. Together with cancer, inflammatory and autoimmune diseases are an important focus for companies developing antibody therapies. In patients with rheumatoid arthritis (RA), TNF-α accumulates in the joints and contributes to the inflammation and joint destruction that is associated with the disease. Marketed products directed towards the regulation of TNF-α include the soluble TNF-α receptor etanercept (Enbrel; Amgen Inc/Wyeth) and the antibody infliximab

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Table 1 | **Approved monoclonal antibodies**

Product	Year approved	Type of molecule	Disease indication	Company
OKT-3	1986	Murine (anti-CD3)	Organ transplant rejection	Ortho Biotech Products LP
ReoPro	1994	Chimeric (anti-platelet gpIIb/IIIa)	Coronary intervention and angioplasty	Centocor Inc
Panorex (Germany only)	1995	Murine (anti-EpCAM)	Colorectal cancer	Centocor Inc
Rituxan	1997	Chimeric (anti-CD20)	Non-Hodgkin's lymphoma	IDEC Pharmaceuticals Corp
Zenapax	1997	Humanized (anti-IL-2 receptor)	Refractory unstable angina	Centocor Inc
Herceptin	1998	Humanized (anti-ERBB2)	Metastatic breast cancer	Genentech Inc
Remicade	1998	Chimeric (anti-TNF-α)	Crohn's disease	Centocor Inc
Simulect	1998	Chimeric (anti-IL-2 receptor)	Kidney transplant rejection	Novartis AG
Synagis	1998	Humanized (anti-F-protein)	Respiratory syncitial viral disease	MedImmune Inc
Mylotarg	2000	Humanized (anti-CD33)	Chemotherapy for acute myeloid leukemia	Celltech Group plc/Wyeth
Campath	2001	Humanized (anti-CD52)	B-cell chronic lymphocytic leukemia	Millennium Pharmaceuticals/ Ilex Oncology Inc.
Zevalin	2002	Mouse (anti-CD20)	B-cell non-Hodgkin's lymphoma	IDEC Pharmaceuticals Corp
Xolair (Australia only)	2002	Humanized (anti-IgE Fc)	Allergy	Tanox Inc/Genentech Inc/ Novartis AG

Adapted and updated from REF. 81.

(Remicade; Centocor Inc). Infliximab is a chimeric antibody with mouse variable domains and human constant domains of the IgG1 subclass. There are a number of anti-TNF-α antibodies in clinical trials, including CDP571 and CDP870 (Celltech Plc) and adalimumab (D2E7; Abbot Laboratories/Cambridge Antibody Technology). Adalimumab is the first phage-display-derived human antibody brought into the clinic, and was generated by 'guided selection' using a mouse monoclonal antibody[28]. The method is based on the selection of a human variable-domain repertoire coupled to one of the original mouse variable domains, so as to 'guide' the human variable-domain repertoire towards the same specificity as the original mouse variable domains. The antibody is affinity optimized by iterative rounds of selection and mutagenesis (FIG. 3; BOX 2, 3). Adalimumab has completed Phase III clinical trials and is currently in registration for FDA approval. Recently, the anti-CD20 antibody rituximab (Rituxan; IDEC Pharmaceuticals; see below) completed Phase II clinical trials in RA and showed promising results compared with the anti-TNF-α antibodies adalimumab and infliximab[29], indicating that depletion of B cells might be an effective treatment in RA and other autoimmune diseases[30]. Eculizumab (5G1.1; Alexion Pharmaceuticals) is a humanized monoclonal antibody that prevents the cleavage of human complement component C5 into its pro-inflammatory components[31], whereas J695 (Cambridge Antibody Technology) is a human antibody derived from a phage-display library against interleukin-2 (see online links box). Both antibodies are in clinical trials at present and have shown potential in the treatment of inflammatory diseases such as RA and nephritis.

Antibodies targeting cancers

In cancer therapy, the purpose of antibody administration is to induce the direct or indirect destruction of cancer cells, either by specifically targeting the tumour or the vasculature that nourishes the tumour. Indeed, new technologies for the panning of antibody libraries on intact cells have made it possible to isolate antibodies against novel and promising cancer-associated antigens[32]. However, the most common cancer targets are the carcinoembryonic antigen (CEA), which is associated with colorectal cancers, MUC1, epidermal growth factor receptor (EGFR) and ERBB2 (also known as HER2/neu, associated with lung and breast cancer) and CD20 on B cells, which is a marker for non-Hodgkin's lymphoma (NHL). Examples of approved therapeutic antibodies directed towards ERBB2 and CD20 include the humanized IgG1 antibody trastuzumab (Herceptin; Genentech) for breast cancer and the chimeric IgG1 antibody rituximab (Rituxan; IDEC Pharmaceuticals) for NHL.

Today, specific antibody therapy is used in combination with classical chemotherapy, but the remaining challenge is to develop specific and highly cytotoxic drugs against cancer cells. Recently, the FDA approved two new antibody chemotherapies: ibritumomab tiuxetan (Zevalin; IDEC Pharmaceuticals) and gemtuzumab ozogamicin (Mylotarg; Wyeth). Ibritumomab tiuxetan is a mouse anti-CD20 antibody

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attached to the radioisotope ^{90}yttrium that targets the surface of the mature B cells and B-cell tumours, inducing cellular damage in the target and neighbouring cells. Ibritumomab tiuxetan is the first radioimmunotherapeutic antibody approved by the FDA and is approved for use in the treatment of NHL. The antibody reacts with the same antigen as rituximab, but is a mouse antibody, which results in beneficial rapid clearance to decrease the undesired effect of total body irradiation. Gemtuzumab ozogamicin is a humanized monoclonal antibody that is linked to the antitumour agent calicheamicin, a bacterial toxin[33]. The antibody is targeted to CD33, which is expressed in about 90% of all acute myelogenic leukaemia (AML) cases, and has been approved for administration to patients who have relapsed AML. Both ibritumomab tiuxetan and gemtuzumab ozogamicin are examples of antibodies designed to specifically deliver their toxic load directly to cancer cells. Antibodies directed against major histocompatibility complex (MHC) class II proteins specifically target and eliminate cancer cells. The mouse monoclonal antibody Oncolym (Lym-1; Peregrine Pharmaceuticals)[34] and the antibody Hu1D10 (Remitogen; Protein Design Labs)[35] are two examples of such antibodies. Whereas one form of Oncolym is radiolabelled with ^{131}iodine (REF. 36), the humanized IgG1 Hu1D10 eliminates cancer cells by virtue of its natural effector functions (FIG. 4 and see below), and also induces apoptosis. Recently, an anti-MHC class II human antibody derived from an antibody phage-display library was shown to induce apoptosis of activated lymphoid cells[19]. Vascular endothelial growth factor (VEGF) is a potent cytokine for angiogenesis (the formation of blood vessels). Inhibitory agents, including specific antibodies, have been developed to block VEGF-stimulated angiogenesis as a strategy to inhibit tumour growth. One example of such an antibody is the humanized anti-VEGF antibody bevacizumab (Avastin; Genentech), which is in Phase III clinical trials for the treatment of breast cancer and colorectal cancer. Monoclonal antibodies are being continuously developed for cancer therapy, and most new antibody formats have demonstrated utility within this field.

Naked antibodies

Both trastuzumab and rituximab are 'naked antibodies', meaning that they do not have a radioisotope or toxin attached to them. The elimination of the target of these antibodies depends entirely on the recruitment of the body's own effector mechanisms, namely complement

Table 2 | **A selection of antibodies in clinical development**

Product	Stage (2002)	Type of molecule	Disease indication	Company
ABX-EGF	Phase II	Human (anti-EGF-R)	Non-small-cell lung cancer	Abgenix Inc/Immunex Corp
ABX-IL8	Phase II	Human (anti-IL-8)	Pulmonary disease, chronic obstructive bronchitis	Abgenix Inc/Immunex Corp
Eculizumab (5G1.1)	Phase IIb	Humanized (anti-C5)	Rheumatoid arthritis, lupus nephritis	Alexion Pharmaceutical Inc
D2E7	Phase III	Human (anti-TNF-α)	Rheumatoid arthritis	Cambridge Antibody Technology/ Abbot Laboratories
CAT-152	Phase II/III	Human (anti-TGF-β2)	Scarring following glaucoma surgery	Cambridge Antibody Technology
J695	Phase II	Human (anti-IL-2)	Autoimmune diseases including rheumatoid arthritis	Cambridge Antibody Technology/Abbot/ Wyeth Genetics Institute
Antegren (natalizumab)	Phase III	Humanized (anti-α-4 integrin)	Crohn's disease, multiple sclerosis, inflammatory bowel disease	Elan Pharmaceuticals Corp/Biogen Inc
Avastin (rhuMAb VEGF)	Phase III (anti-VEGF)	Humanized	Metastatic breast cancer, non-small-cell lung cancer	Genentech Inc
IDEC-151 (Clenoliximab)	Phase II	Primatized (anti-CD4)	Rheumatoid arthritis	IDEC Pharmaceuticals Corp
MEDI-507 (Siplizumab)	Phase II	Humanized (anti-CD2 Receptor)	Suppresses NK and T-cell function	MedImmune Inc
XTL-001 (pairs of monoclonals)	Phase I/II	Human (anti-HBV)	Hepatitis B virus neutralization	XTL Biopharmaceuticals Ltd
CDP870	Phase II	Fab fragment (anti-TNF-α)	Rheumatoid arthritis	Celltech Group plc
CDP571	Phase III	Humanized (anti-TNF-α)	Crohn's disease	Celltech Group plc
Hu1D10 (Remitogen)	Phase II	Humanized (anti-MHC class II)	Non-Hodgkin's Lymphoma	Protein Design Labs Inc

Adapted from REFS 34, 81 and selected company web sites.

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Box 2 | ***in vitro* human antibody libraries**

The strategy of selecting antibodies from large repertoires is dependent on the coupling between genotype and phenotype — that is, the displayed protein (the antibody) carries its own encoding gene. As a result, the process enables the easy recovery of the DNA encoding the selected protein. The power of such antibody libraries lies in the fact that selection takes place *in vitro*, thereby making them excellent systems for isolating antibodies to certain drugs, potent toxins and haptens that would normally be impossible to raise within *in vivo* systems such as the mouse hybridoma technology.

In phage display, the variable genes encoding the antibody variable domains are fused to genes encoding bacteriophage coat proteins. The most commonly used phage protein is the pIII protein located at the tip of the long, thin filamentous phage M13. The system is highly effective and is used to isolate single-chain Fv or Fab fragments with specificity to almost any kind of antigen. The plasmid encoding the variable genes is packaged within the viral capsid and the expressed antibody is presented on the bacteriophage surface. Phage libraries are made from the human naive repertoire (that is, the IgM and IgD class) from pre-immunized donors or totally synthetic variable genes. As the repertoire is created *in vitro*, the technology also generates high-affinity human antibodies against human proteins. The process is based on panning the library against an immobilized target in a test tube (FIG. 3a). The non-binding phage antibodies are washed away and the recovered antibodies are amplified by infection in *E. coli*. The selection rounds are subsequently repeated until the desired specificity is obtained.

In messenger RNA (mRNA)-display technologies, such as the ribosomal display technology, antibody domains are linked to ribosomes attached to the mRNA that they are translating. This process takes place entirely *in vitro* by transcripton of a DNA library followed by *in vitro* translation of the mRNA library. The subsequently recovered mRNA transcripts are reverse transcribed and amplified by polymerase chain reaction (PCR) for the subsequent round of selection. The method has been successfully used for the affinity maturation and stability engineering of antibody fragments[58,70]. A similar technology called ProFusion is based on the covalent linkage of the synthesized proteins to the mRNA template via a puromycin linker[71]. Another potential display method applicable for antibody display is the Covalent Display Technology. In this method, the protein is fused to P2A, a bacteriophage DNA-nicking protein that covalently binds its own DNA[72].

activation and Fc-receptor-dependent responses (FIG. 4). Such effector functions can be abolished or augmented by mutagenesis of the Fc part of the antibody. Both trastuzumab and rituximab seem to exert their effects primarily through an Fc-receptor-dependent mechanism[37], and both are of the IgG1 subclass of antibodies, and thus bind all known Fcγ receptors (FcγRs). These receptors are located in the membrane of various effector cells, such as natural-killer (NK) cells, neutrophils, monocyte/macrophages, dendritic cells and B cells. In general, IgG-FcγR crosslinking leads to antibody-cell cytotoxicity or phagocytosis of the target cells by the effector cell (FIG. 4). In addition, depending on the nature of the target, the crosslinking caused by FcR interaction can itself induce signalling that leads to apoptosis. Although most of the FcγRs exert the functions mentioned above, one class of FcγRs, FcγRIIb, has a general inhibitory effect. Recently, a thorough investigation of the interaction between IgG and FcγRs led to the discovery of IgG1 variants with improved or decreased binding affinity for the FcγRs, including some with increased binding affinity for the activating receptors and decreased binding to the inhibiting ones[38]. So, variants can now be selected with desired combinations of functions.

Another approach to enhance the effector functions of antibodies is to engineer bispecific antibodies, which comprise two specificities, one for the cell to be eliminated and one for receptors on effector cells such as cytotoxic T cells (BOX 4). Strategies for the production of bispecific antibodies have been developed[39], and an important new approach in this field is the activation of polymorphonuclear leucocytes (PMNLs), the most numerous cytotoxic effector-cell population. These cells have Fc receptors for the antibody class IgA (Fcγ receptor, CD89) and this has elicited a growing interest in IgA[40], and bispecific antibodies that recruit PMNLs through CD89 binding have shown promise in *in vitro* studies[41].

A possible side effect of antibody therapy is the cytokine-release syndrome that is probably mediated through recruitment of the immune effector cells.

Box 3 | **Affinity maturation of antibodies**

In the mammalian immune system, antibodies undergo affinity maturation and through the development of memory B cells retain an antibody repertoire for subsequent encounter of an identical pathogen. Similarly, *in vitro* antibody technologies have enabled the selective alteration of the affinity of antibodies. Several approaches have been developed, but a common element to all of these is that they are developed under stringent selection regimes, including reducing the concentration of antigen, altering washing conditions (see BOX 2), changing temperature or eluting with free antigen. Methods of antibody affinity maturation are based on the principle of changing parts of, or the whole, antibody variable domains while simultaneously keeping the specificity. Most of the contribution of specific antibody binding resides within the variable heavy-chain domain and especially within the CDR 3 loops of both variable heavy and variable light chains (see FIG. 1).

Chain-shuffling is based on the method of substituting the native light chain with a new light-chain repertoire, but retaining the variable heavy chain. Panning of such chain-shuffled libraries in stringent conditions generates antibodies with higher affinities than the mother antibody clone. The randomization of CDR regions has also been used as a maturation strategy. Another approach is to generate an antibody library with mutations within the variable genes either by error-prone polymerase chain reaction, *E. coli* mutator strains or site-specific mutagenesis. Both phage display and ribosomal display have been successfully used for affinity maturation.

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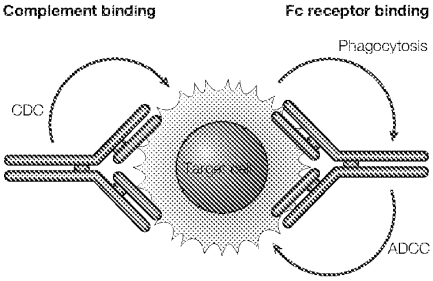

Figure 4 | **Natural immunoglobulin effector functions.** The classical complement pathway is initiated by the binding of C1 to immune complexes (that is, antibody-coated cells or antigens) and eventually leads to cellular lysis and elicits inflammatory responses for the ultimate elimination of the target cell. This process is called complement-dependent cytotoxicity (CDC). The Fc-receptor-mediated effector functions are associated with the recruitment of cellular effectors leading to either phagocytosis or antibody-dependent cellular cytotoxicity (ADCC). These mechanisms are accepted to be the processes that operate when naked antibodies are used as therapeutic agents.

In such cases, abrogation of Fc-dependent effector functions is desired and this can be achieved by mutating single amino acids in the Fc region of the antibodies[42].

Antibodies without Fc

Small antibody-derived fragments can do part of the job normally performed by intact antibodies, such as blocking the action of a toxin — for example, anthrax toxin[23] — or blocking the interaction between a cytokine and its receptor[43,44]. These antibody fragments can also carry effector molecules to their targets. The blocking effect of antibodies simply requires the inhibition of subsequent ligand or receptor binding, and does not necessarily need effector functions. However, even fragments with effector functions normally associated with 'naked antibodies' — the bispecific antibody fragments — have been described. These fragments comprise either the Fv or Fab portion of the antibody (FIG. 1), which in both cases is the part that binds antigen. Such antibody fragments can be functionally expressed in *E. coli* with ease, which is a prerequisite for antibody phage display as well as for other *in vitro* human antibody library approaches. One of the great advantages of the library approaches in general is that they allow for the selection of antibodies of high specificity and affinity towards a variety of different antigens (BOX 2). Engineered antibodies have therefore become smaller, with increasing affinity and specificity. Another obvious advantage is the ability to select specific antibodies against toxins, drugs, cytokines and other targets that cannot, for various reasons, be injected into an animal to raise an immune response, one of which is that the target might kill the animal or that it might not be immunogenic at all (BOX 2).

Coupling the antibody fragments into dimers and trimers generates oligovalent antibody fragments with increased avidity and the ability to crosslink target molecules (FIG. 5). In this approach, the antibody is first reduced to the small antigen-binding portion and then engineered to form a larger molecule to gain avidity and crosslinking ability. So, the signalling effect that requires crosslinking of receptors might also be achieved with antibody fragments. The so-called triabodies are antibody fragments in which the valency is increased to three antigen-binding sites[45,46], thereby increasing the avidity of the antibody fragments. Such multivalent antibody fragments might prove to be effective in the blocking and neutralization of toxins, as well as in the generation of targeting devices for cancer therapy.

Antibody fragments with effector functions normally associated with 'naked antibodies' — the diabodies — were first described in 1993 (REF. 47). Diabodies are scFv fragments with two different antigen specificities, one directed against the target and one directed against effector molecules such as complement[48] or Fc receptors[49]. Since then, diabodies have been designed in various ways to crosslink effector cells such as cytotoxic T cells[50,51] and NK cells[49], which is particularly useful for the treatment of lymphomas. Tandem diabodies[52], in which two diabodies are genetically coupled to achieve a better crosslinking effect, are still at an early stage of development. Another early-stage antibody format is pepbodies[53], which comprise Fab or scFv fragments linked to small peptides that bind effector molecules and thus mimic the way complete antibodies bind their effector molecules.

Antibody pharmacokinetics

A prerequisite for effective antibody targeting is that the antibodies should be able to penetrate tissues, and small antibody fragments are better than complete antibodies in this respect. Recent research has shown that high-affinity fragments are retained in the periphery of the tumour, whereas the medium-affinity antibodies penetrate throughout the tumour[54]. Furthermore, bivalent low-affinity fragments penetrate better and more uniformly than high-affinity fragments[55]. Fortunately, antibody fragments can be selected for optimal affinity and specificity by all of the *in vitro* selection processes[56,57]. The therapeutic efficacy of an antibody, however, also depends on its stability as well as its immunogenicity. Strategies for the engineering of antibody stability with regards to antibody fragments have been described[58].

Box 4 | **Strategies for making bispecific antibodies**

Bispecific antibodies have the ability to bind two different antigens and have been used to crosslink various cells and molecules, although they have been primarily used to re-direct effector cells to target cells (FIG. 5). Methods for generating bispecific antibodies tend to bias the production towards heterodimers — that is, towards two specificities instead of one. The first method was described in 1983 (REF. 73) in the generation of so-called hybrid-hybridomas, or quadromas, and is based on the fusion of two hybridoma cell lines, but this is a complex and time-consuming effort; chemical or genetic coupling of Fab fragments[74] is more effective. The inclusion of a C_H3 domain into antibody fragments has been used to function as a dimerization domain, and a modified form of a C_H3 domain has been used to direct hetero-dimerization[59]. Perhaps the most effective way to generate bispecific antibody fragments is by expression of bispecific single-chain Fv fragment as diabodies (FIG. 5).

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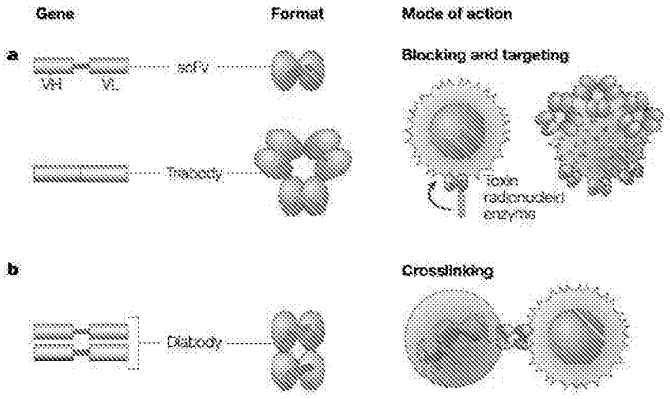

Figure 5 | **Different formats of antibody fragment. a** | Single-chain Fv fragments (scFvs) are mostly monomeric when the peptide linker (in red) between the V_H and the V_L domains is about 12–15 amino acids. When the linker is shorter than three amino acids or is totally deleted, the fragments tend to make trimeric fragments or even tetrameric formats. Multivalent antibodies gain avidity and such molecules are therefore perfect for blocking viral infection or receptor–ligand interactions, for example. **b** | If the linker is about five amino acids long, then the variable domains tend to dimerize. When equipped with variable domains with different specificities, such dimeric scFvs can make bispecific fragments called diabodies.

Similarly, the presumed immunogenicity of human antibodies can be reduced by mutagenesis of typical B-cell[59] or T-cell epitopes[60]. As the development of monoclonal antibodies moved away from mouse antibodies to chimeric antibodies and subsequently to humanized antibodies, a reduction in the level of anti-antibody responses was observed with new antibody products. It will, however, be interesting to observe the immunogenicity of fully human antibodies in therapeutic settings. In particular, a comparison of D2E7 with infliximab (that is, a human versus a chimeric anti-TNF-α monoclonal antibody) will provide valuable information in this respect. Small antibody fragments show rapid clearance from the circulation, and as a result the fraction of the injected dose that reaches its target is at present too low for a therapeutic benefit, even for bivalent fragments. Therefore, the manipulation of antibody fragments to increase their half-life will be crucial for the success of therapeutic antibody fragments.

Complete human antibodies have long serum half-lives owing to their ability to bind to the so-called neonatal Fc receptor (FcRn). This receptor both transports IgG across the placenta from mother to fetus and protects serum IgG from degradation. FcRn is expressed on placenta and blood-vessel linings and binds IgG in the junction between the C_H2 and C_H3 domains of the Fc region[61] (see FIG. 1). As Fab and scFv fragments lack the Fc region altogether, they are not rescued from degradation by this mechanism and, as a result, have a short serum half-life.

A new approach to increasing the half-life of antibodies is the PEGYLATION of antibodies and antibody fragments, which simultaneously reduces their immunogenicity[62]. The effect is achieved by chemical coupling of polyethylene glycol (PEG) to amino groups in the protein structure. The main property contributing to this effect is the increase in the size of the molecule above the glomerular-filtration limit. However, modifications of amino groups within antibodies affect the effector functions[63] as well as the antigen-binding capacity[64]. A possible alternative is to direct the PEG attachment to sites on the antibody molecules that are distant from the CDR regions and crucial sites for effector functions[62]. Indeed, an anti-TNF-α human Fab fragment (CDP 870; Celltech) has its circulating half-life prolonged to 14 days by site-specific pegylation in the hinge region[44].

PEGYLATION
The covalent attachment of the polymeric molecule polyethylene glycol (PEG) to proteins.

TROYBODY
An antibody with specificity for an antigen-presenting cell and with an antigenic fragment inserted into a constant domain.

The potentiation of immune responses

Antibodies are the perfect high-specificity targeting and delivery devices. However, in the immune responses mounted by the body in response to pathogenic organisms and cancer, the cellular response mediated by T cells is a crucial component. Infectious diseases are eliminated by the combined action of antibodies and T cells. In the search for optimized vaccines, the aim is to use agents that both stimulate antibody and T-cell responses. A discussion of the immune effects of T cells is beyond the scope of this review, although new antibody-based technologies that aim at modulating T-cell responses are important issues for the potentiation of a desired therapeutic effect by, for example, therapeutic antibodies. One strategy for increasing the immune response is to load antibodies with antigenic material in the CDR regions[65]. This strategy is one of several that aim to use the Fc portion of the antibody to load specialized immune cells with antigenic material. In the TROYBODY strategy[66], the antibodies carry pieces of antigenic material as part of their Fc region and target the specialized immune cells by way of their Fab regions. In all cases, the purpose of the immune-cell loading is to activate disease-specific helper T cells. Indeed, Troybodies increase such T-cell responses more than 10,000-fold compared with the administration of the antigenic material in free form[67,68]. This strategy requires that the antigenic material is characterized, which is a significant challenge. An advantage of this approach, however, is that it can be combined with strategies that aim at activating the cytotoxic T cells that are crucial in the destruction of cancer and virus-infected cells. Recently, myeloma cells were coated with antibodies specific for syndecan-1 (SDC1), an antigen that is highly expressed on these tumour cells, which promoted the uptake of the tumour cells by dendritic cells through an Fc-receptor-dependent mechanism and the subsequent stimulation of tumour-specific cytotoxic T cells[69].

Concluding remarks

Throughout the development of therapeutic antibodies over the past 30 years, many obstacles have been overcome and, today, therapeutic antibodies have entered a new era. It is no longer a significant effort to select human antibodies against any kind of disease target. The first antibody derived from phage antibody

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libraries has recently completed Phase III trials, and there are, at the moment, several other antibodies derived from both *in vitro* libraries and transgenic mice in clinical trials. There is a rising awareness of antibody effector functions and efforts are being directed towards modulating Fc-associated functions, such as complement activation and Fc-receptor activity. A remaining impediment to the development of new antibody therapeutics by the pharmaceutical industry is the cost of producing antibodies with mammalian expression systems. The new small therapeutic antibody formats, such as scFv, Fab or diabodies, can, however, be produced in *E. coli* at a much lower cost, and even antibody production in plants is a growing field. As antibodies are such versatile and highly specific molecules, fine tuned by millions of years of evolution to be optimal agents for targeted disease elimination, their development for various indications will, no doubt, be pursued with vigour for the foreseeable future.

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Abstract

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Linking outcomes management to quality-of-life measurement.

Cella D[1], Webster K.

Author information

Abstract

Health-care workers now accept quality of life (QOL) as an important outcome to evaluate in clinical research and as a useful measure of quality care. Indeed, current demand for QOL **assessment** in clinical practice has outpaced the availability of valid, streamlined, cost-effective methods for carrying out such **assessment**, although new tools are in the offing. This paper will highlight some of the major challenges facing outcomes management and outcomes research, with particular focus on the development of a QOL instrument to evaluate and manage **anemia** and **fatigue** in cancer patients--the **Functional Assessment** of Cancer **Therapy-Anemia** (FACT-An). The newest version of broader QOL **assessment** system, the **Functional Assessment** of **Chronic Illness Therapy** (FACIT), will also be described.

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eculizumab Alexion clinical data (phase III) (PNH)

Publication info: R & D Focus Drug News (Feb 6, 2006): NA.
ProQuest document link

Abstract (English): On 26 January 2006 Alexion reported data from a phase III trial of eculizumab (SOLIRIS), a humanized monoclonal antibody targeted to complement C5, in the treatment of paroxysmal nocturnal hemoglobinuria (PNH). The double-blind, randomized,
placebo-controlled, multicenter phase III trial, designated TRIUMPH, was conducted in the USA, Canada, Europe and Australia. The study evaluated the effects of eculizumab on hemoglobin stabilization and blood transfusion in hemolytic,
transfusion-dependent PNH patients during six months of therapy. The primary endpoints were median transfusion rate and hemoglobin stabilization over six months. Results showed that the median transfusion rate was reduced from 10 units/patient for
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placebo, to 0 units/patients for eculizumab (p is less than 0.000000001). Hemoglobin stabilization was achieved by 49% of eculizumab-treated patients compared with 0% of placebo-treated patients (p is less than 0.0000001). European and US regulatory
submissions for eculizumab in the treatment of PNH are planned for the second half 2006.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, SOLIRIS, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5B, Systemic Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All
Other Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion, clinical data (phase III)

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eculizumab Alexion clinical data (PNH)

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Abstract (English): On 12 December 2005, Alexion reported an analysis of three-year cumulative data from an open-label extension study of eculizumab in ten patients with paroxysmal nocturnal hemoglobinuria (PNH). Results showed continued treatment with eculizumab
maintained the reduction in intravascular hemolysis observed in a 12-week pilot study. A sustained and stable increase in PNH red blood cell counts (from 1.37 x10(12)/L to 2.37 x10(12)/L at maximum response) was also seen following three-year chronic
treatment with the agent. The levels of lactate dehydrogenase decreased from a mean of 3233 (+/- 648) during the 12 months before treatment to 656 (+/-37) following eculizumab treatment for at least 36 months (p=0.004). The increase in PNH red blood cells

Full text: On 12 December 2005, Alexion reported an analysis of three-year cumulative data from an open-label extension study of eculizumab in ten patients with paroxysmal nocturnal hemoglobinuria (PNH). Results showed continued treatment with eculizumab
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coincided with reductions in the mean and median transfusion rates from 2.1 and 1.8 units/patient/month during the 12 months prior to treatment, to 0.3 and 0.3 units/patient/month (p=0.002) following 36-month eculizumab therapy, respectively. Treatment
with eculizumab alone or in combination with erythropoietin in aplastic patients resulted in seven of the ten patients becoming transfusion independent for at least the last 17 months of the study. Eculizumab was well tolerated; the most common adverse
events reported were headaches, upper respiratory tract infections and nausea. ....Alexion expects to report top line results from a double-blind, randomized, placebo-controlled multicenter phase III trial, designated TRIUMPH, in the first quarter 2006.
The trial evaluated the effects of eculizumab on hemoglobin stabilization and blood transfusion in approximately 75 hemolytic, transfusion-dependent PNH patients during six months of therapy. Eculizumab, a humanized monoclonal antibody targeted to
complement C5, is also undergoing an open-label, non-placebo-controlled, multicenter phase III safety study in hemolytic PNH patients with a history of transfusion.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5B, Systemic Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All Other
Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion, clinical-data

Subject: Rheumatoid arthritis

Identifier (keyword): Trade

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Jan 30, 2006

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Magazine/Journal

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eculizumab Alexion completes enrollment

Publication info: R & D Focus Drug News (Feb 17, 2003): NA.
ProQuest document link

Abstract (English): Alexion has completed enrollment in a phase IIb trial of eculizumab, its humanized monoclonal antibody targeting complement C5, in the treatment of rheumatoid arthritis. Approximately 350 patients with rheumatoid arthritis are enrolled in the study,
which will assess safety and efficacy of the monoclonal, and will confirm an effective dosing regimen. In the randomized, double-blind, placebo-controlled trial, patients are to receive either placebo, iv eculizumab once weekly for four weeks then once
monthly, or iv eculizumab once weekly for four weeks followed by once every two weeks. After six months, patients are to be evaluated for safety and efficacy; the primary endpoint is improvement in ACR 20 score.

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eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5, Nonsteroidal Products for Inflammatory Skin Disorders Including Psoriasis, M5X, Other Musculoskeletal
Products, G4B9, All Other Urological Products, D11A, Other Dermatologicals, Alexion

Subject: Care and treatment;Monoclonal antibodies -- Product development;Patient care;Patients;Patients -- Care and treatment;Pharmaceutical industry -- Product development;Product development;Rheumatoid arthritis -- Care and treatment

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.; Alexion Pharmaceuticals Inc. (Product development)

Identifier (keyword): Trade, ALXN

Event classification: 331: Product development

Alternate title: in a phase IIb trial

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Feb 17, 2003

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Brief Article

Source attribution: Gale Health and Wellness, © Publisher specific

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eculizumab Alexion completes enrollment for phase III PNH trial

Publication info: R & D Focus Drug News (Apr 25, 2005): NA.

ProQuest document link

Abstract (English): Alexion announced on 12 April 2005 that it has completed enrollment of more than 75 patients for its phase III TRIUMPH trial of eculizumab in patients with paroxysmal nocturnal hemoglobinuria (PNH). The trial aims to evaluate the effects of eculizumab
on hemoglobin stabilization and blood transfusion in transfusion-dependent PNH patients during six months of therapy. All patients are expected to have completed the six-month treatment phase by the end of 2005. In addition, a second phase III trial,
designated SHEPHERD, is under way to provide additional safety data for eculizumab in hemolytic PNH patients with a history of transfusion. ....Eculizumab, a humanized monoclonal antibody targeted to complement C5, may also have potential in the treatment

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designated SHEPHERD, is under way to provide additional safety data for eculizumab in hemolytic PNH patients with a history of transfusion. ....Eculizumab, a humanized monoclonal antibody targeted to complement C5, may also have potential in the treatment
of autoimmune diseases such as rheumatoid arthritis and other inflammatory disorders.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5A, Topical Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All Other
Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion

Subject: Clinical trials;Pharmaceutical industry -- Product development;Product development

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.; Alexion Pharmaceuticals Inc. (Product development)

Identifier (keyword): Trade, ALXN

Event classification: 331: Product development

SIC classification: 2834: Pharmaceutical preparations

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Apr 25, 2005

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Brief Article

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eculizumab Alexion initiates second phase III study

Publication info: R & D Focus Drug News (Jan 17, 2005): NA.
ProQuest document link

Abstract (English): Alexion announced on 10 January 2005 that it has initiated a second phase III trial, designated SHEPHERD, to provide additional safety data for eculizumab in the treatment of paroxysmal nocturnal hemoglobinuria (PNH) in hemolytic patients with a
history of transfusion. The open-label, non-placebo-controlled, multicenter trial aims to enroll up to 75 patients at centers in the USA, Canada, Europe and Australia. Initial results from the trial are expected second half 2005. A phase III trial,
designated TRIUMPH, initiated in November 2004 and is evaluating the effects of eculizumab on hemoglobin stabilization and blood transfusion in approximately 75 hemolytic, transfusion-dependent PNH patients during six months of therapy. The SHEPEHRD and

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designated TRIUMPH, initiated in November 2004 and is evaluating the effects of eculizumab on hemoglobin stabilization and blood transfusion in approximately 75 hemolytic, transfusion-dependent PNH patients during six months of therapy. The SHEPEHRD and
TRIUMPH trials will provide the basis of a BLA filing. Alexion received Orphan Drug designation for eculizumab in the treatment of PNH from the US FDA and the EMEA in December 2003. Eculizumab, a humanized monoclonal antibody targeted to complement C5, is
also being developed for the treatment of idiopathic membranous glomerulonephritis and dermatomyositis.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5A, Topical Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All Other
Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion

Subject: Antirheumatic agents -- Licensing, certification and accreditation;Licensing, certification and accreditation;Pharmaceutical industry -- Product development;Product development

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.; Alexion Pharmaceuticals Inc. (Product development)

Product name: eculizumab (Medication) (Licensing, certification and accreditation)

Identifier (keyword): Trade, ALXN

Event classification: 331: Product development;930: Government regulation

SIC classification: 2834: Pharmaceutical preparations

Alternate title: Alexion Pharmaceuticals Inc

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Jan 17, 2005

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Brief Article

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eculizumab Alexion phase change III, USA, Europe Canada, Australia (PNH)

Publication info: R & D Focus Drug News (Nov 15, 2004): NA.
ProQuest document link

Abstract (English): Alexion announced on 1 November 2004 that it has initiated a phase III trial to evaluate its humanized monoclonal antibody targeted to complement C5, eculizumab, in the treatment of paroxysmal nocturnal hemoglobinuria (PNH). The double-blind,
randomized, placebo-controlled multicenter trial, designated TRIUMPH, will evaluate the effect of eculizumab on hemoglobin stabilization and blood transfusion in approximately 75 hemolytic, transfusion-dependent PNH patients during six months of therapy.
The trial is being conducted at sites in the USA, Canada, Europe and Australia. ....A second phase III trial, designated SHEPHERD, is also planned for 2004. This trial aims to provide additional safety data for eculizumab in the treatment of hemolytic PNH

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The trial is being conducted at sites in the USA, Canada, Europe and Australia. ....A second phase III trial, designated SHEPHERD, is also planned for 2004. This trial aims to provide additional safety data for eculizumab in the treatment of hemolytic PNH
patients with a history of transfusion. ....The US FDA approved the design of both trials in July 2004, under the FDA's Special Protocol Assessment process. Alexion plans to submit a BLA to the US FDA for the use of eculizumab for this indication, based
on results from these trials. The compound is also being evaluated for the treatment of autoimmune diseases, such as rheumatoid arthritis.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5A, Topical Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All Other
Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion, phase-III, USA, Europe, Canada, Australia, new-phase

Subject: Care and treatment;Clinical trials;Drugs -- Evaluation;Evaluation;Pharmaceutical industry -- Services; Rheumatoid arthritis -- Care and treatment;Services

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.; Alexion Pharmaceuticals Inc. (Services)

Identifier (keyword): Trade, ALXN

Event classification: 360: Services information

NAICS classification: 325412: Pharmaceutical Preparation Manufacturing;3254: Pharmaceutical and Medicine Manufacturing

Product classification: 2834199: Metabolic Agents NEC;2834190: Metabolic Agents;2830000: Drugs &Pharmaceuticals;2834000: Pharmaceutical Preparations

SIC classification: 2834: Pharmaceutical preparations;2830: Drugs

Alternate title: eculizumab Alexion phase change III, USA, Europe, Canada Australia (PNH)

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Nov 15, 2004

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Brief Article

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eculizumab Alexion plans phase III trials (paroxysmal nocturnal hemoglobinuria)

Publication info: R & D Focus Drug News (Aug 2, 2004): NA.

ProQuest document link

Abstract (English): Alexion reported that the US FDA has approved its protocol of two phase III trials of eculizumab, a humanized monoclonal antibody targeted to complement C, for the treatment of paroxysmal nocturnal hemoglobinuria (PNH), which are planned to commence
during 2004; the TRIUMPH trial aims to examine the effects of eculizumab on hemoglobin stabilization and blood transfusion in hemolytic transfusion-dependent PNH patients during six months of therapy. The double-blind, randomized, placebo-controlled
multicenter TRIUMPH study aims to enroll approximately 75 patients and will be conducted at sites in the USA, Canada, Europe, Australia and New Zealand. ....A second trial, designated SHEPHERD, aims to provide additional safety data of eculizumab in

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hemolytic PNH patients with a history of transfusion. The SHEPHERD study will be an open-label, nonrandomized, nonplacebo-controlled, multicenter trial and aims to enroll approximately 75 patients at sites in the USA, Canada, Europe, Australia and New
Zealand. ....In December 2003 Alexion received Orphan Drug designation for eculizumab in the treatment of PNH from the US FDA and the EMEA. The compound is also being evaluated for the treatment of autoimmune diseases such as rheumatoid arthritis as well
as musculoskeletal disorders and psoriasis.
eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, M1A1, Antirheumatic Non-Steroidal Plain, L4A, Immunosuppressive Agents, D5A, Topical Antipsoriasis and Similar Products, M5X, Other Musculoskeletal Products, G4B9, All Other
Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion

Subject: Pharmaceutical industry -- Product development;Product development

Location: United States

Company / organization: Alexion Pharmaceuticals Inc.; Alexion Pharmaceuticals Inc. (Product development)

Identifier (keyword): Trade, ALXN

Event classification: 331: Product development

SIC classification: 2834: Pharmaceutical preparations

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Aug 2, 2004

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

Journal code: 0FQN

Source type: Magazine/Journal

Language of publication: English

Document type: Brief Article

Source attribution: Gale PROMT, © Publisher specific

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eculizumab Alexion Priority Review, USA (PNH)

Publication info: R & D Focus Drug News (Nov 20, 2006): NA.

ProQuest document link

Abstract (English): Alexion announced on 14 November 2006 that the US FDA has accepted and granted Priority Review designation for its Biologics License Application (BLA) seeking approval of eculizumab (SOLIRIS), in the treatment of paroxysmal nocturnal hemoglobinuria
(PNH). Alexion filed the BLA in September 2006. ....Eculizumab was also submitted for approval for this indication in the EU in September 2006 and is undergoing review through the EMEA's Accelerated Assessment Procedure. Eculizumab is a humanized
monoclonal antibody targeted to complement C5, designed to inhibit complement activation with a prolonged duration of action and therefore with potential in limiting inflammation in chronic autoimmune diseases.

Full text: Alexion announced on 14 November 2006 that the US FDA has accepted and granted Priority Review designation for its Biologics License Application (BLA) seeking approval of eculizumab (SOLIRIS), in the treatment of paroxysmal nocturnal hemoglobinuria
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eculizumab, MAb, C5, MAb, complement C5, h5G1.1, h5G1.1VHC+h5G1.1VLC, SOLIRIS, M1C, Specific Antirheumatic Agents, L4A, Immunosuppressive Agents, D5B, Systemic Antipsoriasis and Similar Products, M5X, All Other Musculoskeletal Products, G4B9, All
Other Urological Products, D11A, Other Dermatologicals, B3X, Other Antianemic Products, Alexion, Priority Review, USA

Subject: Drug therapy;Rheumatoid arthritis -- Drug therapy

Identifier (keyword): Trade

Publication title: R &D Focus Drug News

Pagination: NA

Publication date: Nov 20, 2006

Publisher: IMSworld Publications Ltd.

Journal subject: Business, Pharmaceuticals and cosmetics industries

ISSN: 1350-1135

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Source type: Magazine/Journal

Language of publication: English

Document type: Magazine/Journal

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Eculizumab found to be well tolerated and efficacious during 52 weeks of treatment in PNH

Publication info: ESPICOM Pharmaceutical and Medical Device News (Jan 2, 2007).
ProQuest document link

Full text: At the American Society of Hematology 48th Annual Meeting and Exposition, held in December 2006, results from the global, open-label Phase III SHEPHERD trial were presented. The data showed that Soliris (eculizumab; formerly known as 5G1.1), a novel humanised monoclonal antibody investigational drug developed by Alexion Pharmaceuticals, appeared to be safe and well tolerated, plus provided clinically and statistically significant improvements in intravascular haemolysis, anaemia, fatigue and quality of life in patients with paroxysmal nocturnal haemoglobinuria (PNH) during 52 weeks of treatment. According to investigators in the trial, the improvements made with eculizumab treatment occurred in a diverse and heterogeneous population of patients with PNH, which suggests the potential of the drug in the treatment of this disorder.
During the study, eculizumab was found to be well tolerated, with an adverse event (AE) profile similar to that of placebo-treated patients in the Phase III TRIUMPH study. Further, no serious AEs were deemed probably or definitely related to treatment. The most common AEs reported with eculizumab treatment in SHEPHERD were: headache, nasopharyngitis, nausea and upper respiratory tract infection. These events were generally consistent with those observed during six months of eculizumab and placebo treatment in TRIUMPH.
Eculizumab therapy reduced intravascular haemolysis, as shown by a reduction in the median lactate dehydrogenase area under the curve (-632,264 U/L W day; p<0.001). LDH levels during the study were reduced by 87 per cent, from a median of 2051 U/L at baseline to 269 U/L after 52 weeks of treatment (p<0.001). Clinically and statistically significant improvements in fatigue were observed as measured by change from baseline using the Functional Assessment of Chronic Illness Therapy-Fatigue instrument (p<0.001) and the fatigue scale of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) instrument (p<0.001).
Treatment with eculizumab also significantly improved overall health and patient functioning as measured by EORTC QLQ-C30, including the global health status scale (p<0.001) and all five aspects of patient functioning: role (p<0.001), social (p<0.001), cognitive (p<0.001), physical (p<0.001) and emotional (p<0.001). Treatment also significantly improved seven of nine EORTC QLQ-C30 symptom scales and single-item measures, including pain (p<0.001), dyspnoea (p<0.001), appetite loss (p<0.001) and insomnia (p<0.001).
Clinically and statistically significant improvements in anaemia were also observed with eculizumab therapy, as evidenced by the reduction in transfusion requirements from a median of eight packed red cells in the 12-month pretreatment period to zero units during the 12 months of treatment (p<0.001). Additionally, 51 per cent of patients were transfusion independent for the entire 12-month treatment period (p<0.001). Other improvements in anaemia included a 44 per cent increase in the endogenous PNH red blood cell mass (p<0.001) and an increase in haemoglobin levels from 9.2g/dL at baseline to 10.2g/dL after 12 months of treatment (p<0.001).
Alexion also reported the initiation of an Expanded Access Program for eculizumab in the US for patients with PNH, in accordance with a Treatment Protocol, entitled EMBRACE, which was authorised by the FDA and expected to begin immediately. It is anticipated that treatment in EMBRACE will continue until marketing approval.
Eculizumab, a long-acting C5 complement inhibitor, is designed to selectively block terminal complement activation, thereby preventing the destruction of red blood cells by complement in patients with PNH. There is currently no therapy specifically available for the treatment of PNH.

Subject: Pharmaceutical

Language: English

Document type: Article

Publication title: ESPICOM Pharmaceutical and Medical Device News

Publication type: Newsletter

Publication date: Jan 2, 2007

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First available: 2010-11-15

Database: ESPICOM Pharmaceutical &Medical Device News (2000 - current)

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Inhibition of complement, neutrophil, and platelet activation by an anti-factor D monoclonal antibody in simulated cardiopulmonary bypass circuits

Michael Fung, PhD[a]
Paul G. Loubser, MD[b*]
Akif Ündar, PhD[c,d,e]
Maryann Mueller, CCP[d]
Cecily Sun, MS[a]
William N. Sun, PhD[a]
William K. Vaughn, PhD[e]
Charles D. Fraser, Jr, MD[c,d,e]

Objectives: Patients undergoing cardiopulmonary bypass frequently manifest generalized systemic inflammation and occasionally manifest serious multiorgan failure. Inflammatory responses of bypass are triggered by contact of blood with artificial surfaces of the bypass circuits, surgical trauma, and ischemia-reperfusion injury. We studied the effects of specific inhibition of the alternative complement cascade by using an anti-factor D monoclonal antibody (166-32) in extracorporeal circulation of human whole blood used as a simulated model of cardiopulmonary bypass.

Methods: Five healthy blood donors were used in the study. Monoclonal antibody 166-32 was added to freshly collected, heparinized human blood recirculated in a pediatric cardiopulmonary bypass circuit at a final concentration of 18 μg/mL. An irrelevant monoclonal antibody was used as a negative control with the same donor blood in a parallel bypass circuit on the same day. Blood samples were collected at different time points during recirculation for measurement of activation of complement, neutrophils, and platelets by immunofluorocytometric methods and enzyme-linked immunosorbent assays.

Results: Monoclonal antibody 166-32 inhibited the alternative complement activation and the production of Bb, C3a, sC5b-9, and C5a. Upregulation of CD11b on neutrophils and CD62P on platelets was also significantly inhibited by monoclonal antibody 166-32. This is consistent with the inhibition of the release of neutrophil-specific myeloperoxidase and elastase and platelet thrombospondin. The production of proinflammatory cytokine interleukin 8 was also suppressed by the antibody.

Conclusions: The alternative complement cascade is predominantly activated during extracorporeal circulation. Anti-factor D monoclonal antibody 166-32 is effective in inhibiting the activation of complement, neutrophils, and platelets. Inhibition of the alternative complement pathway by targeting factor D could be useful in reducing systemic inflammation in patients undergoing cardiopulmonary bypass.

From Tanox, Inc[a]; the Departments of Anesthesiology[b] and Surgery,[c] Baylor College of Medicine; Texas Children's Hospital[d]; and Texas Heart Institute,[e] Houston, Tex.

This study was supported by Tanox, Inc, Houston, Tex.

Received for publication Sept 18, 2000; revisions requested Dec 7, 2000; revisions received Jan 23, 2001; accepted for publication Jan 29, 2001.

Address for reprints: Michael Fung, PhD, Tanox, Inc, 10301 Stella Link, Houston, TX 77025 (E-mail: mfung@tanox.com).

*Current affiliation of Paul G. Loubser, MD: McAllen Medical Center, McAllen, Tex.

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doi:10.1067/mtc.2001.114777

Patients undergoing cardiopulmonary bypass (CPB) frequently manifest a generalized systemic inflammatory response syndrome, which may contribute to perioperative tissue injury and impaired hemostasis.[1-6] Serious inflammatory conditions could lead to delayed recovery and occasionally to multiorgan dysfunction.[1,2,7] Complement activation has been implicated as the most important cause of the

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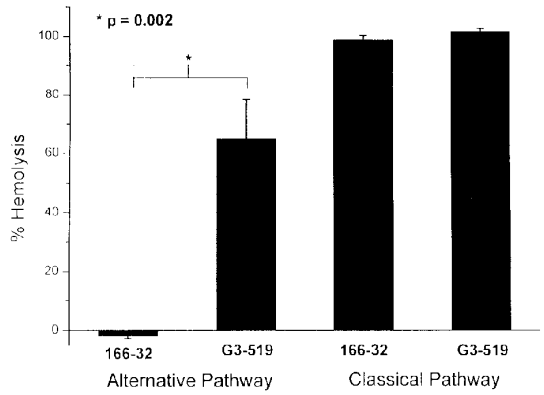

Figure 1. Selective inhibition of the alternative complement activity in human whole blood by MAb 166-32 at 18 μg/mL in the extracorporeal circulation studies before recirculation. MAb 166-32 did not inhibit the classical complement activity of the human plasma in the circuits. The negative control MAb G3-519 had no effects on either the alternative or the classical complement activity. Values are given as means ± SD from 5 paired experiments of the test and control circuits. Percentage of hemolysis was defined as indicated in the text. *P* values were determined by means of paired *t* tests between MAb 166-32– and MAb G3-519–treated circuits in the alternative complement hemolytic assays.

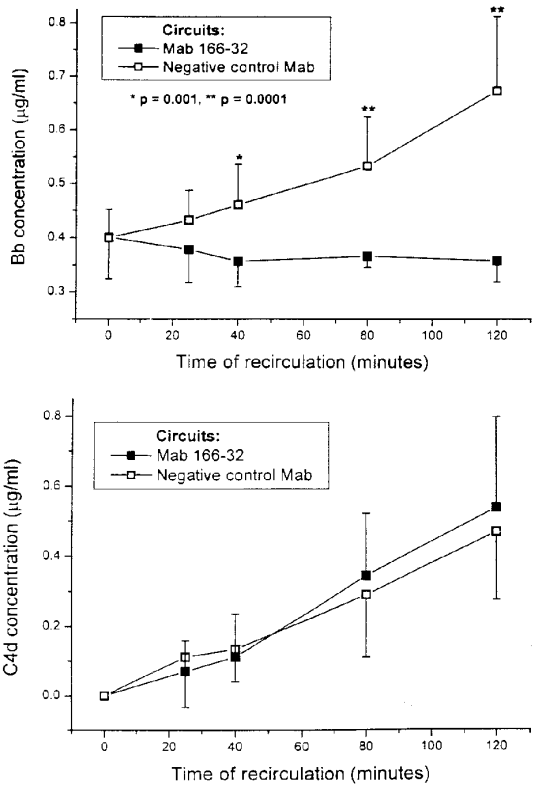

Figure 2. Bb and C4d production in the extracorporeal circuits treated with MAb 166-32 or the negative control MAb G3-519. Bb (A) and C4d (B) were measured by using a quantitative ELISA. Values are given as means ± SD from 5 paired experiments of the test and control circuits. MAb 166-32 completely inhibited the production of Bb, indicating complete suppression of the alternative complement pathway. *P* values were determined by 2-factor analysis of variance. There is no significant difference in the plasma levels of C4d between the test and control circuits as analyzed with 2-factor analysis of variance.

systemic inflammatory reaction.[1,3,4,6,8,9] It is attributed to the interaction between blood and the artificial surfaces of extracorporeal circuits constituting CPB machines.[10,11] Primary inflammatory substances are generated after activation of the complement system, including the anaphylatoxins C3a and C5a, the opsonin C3b, and the membrane attack complex C5b-9.[12] C3a and C5a are potent stimulators of neutrophils, monocytes, and platelets.[13-16] Activation of these cells results in release of proinflammatory cytokines (eg, interleukin 1 [IL-1], IL-6, IL-8, and tumor necrosis factor α), oxidative free radicals, and proteases.[17-22] C5a has been shown to upregulate adhesion molecules CD11b (α-integrin) and CD18 (β_2-integrin) of Mac-1 in polymorphonuclear cells (PMNs; comprising mainly neutrophils) and to induce degranulation of PMNs to release proinflammatory enzymes.[15,16,23-27] C5b-9 induces the expression of adhesion molecule P-selectin (CD62P) on platelets,[28] whereas both C5a and C5b-9 induce surface expression of P-selectin on endothelial cells.[29] These adhesion molecules are involved in the interaction among leukocytes, platelets, and endothelial cells. The expression of adhesion molecules on activated endothelial cells is responsible for sequestration of activated leukocytes, which then mediate tissue inflammation and injury.[24,29,30] In addition, C3a and C5a stimulate chemotaxis of human mast cells and trigger the release of histamine, proteases, and leukotrienes, which induce vascular permeability, vasoconstriction, and tissue inflammation.[31,32]

Factor D is the rate-limiting enzyme important for the activation and amplification of the alternative complement pathway.[33,34] Inasmuch as the concentration of factor D in human blood is among the lowest of all soluble complement components (approximately 2 μg/mL),[33] factor D is a potential target for inhibiting the activation of the alternative complement cascade associated with inflammatory responses. Previously, specific inhibition of the alternative complement pathway was demonstrated with monoclonal antibodies (MAbs) to human factor D with moderate activity in complement-mediated hemolytic assays.[35] Polyclonal rab-

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bit antibodies to mouse factor D were shown to inhibit alternative complement activity in mice.[36] We have generated a potent and specific MAb to human factor D, designated 166-32. The antibody inhibits human factor D at a molar ratio of about 1:2 in complement-mediated hemolytic assays.[37] The antibody was shown to protect isolated rabbit hearts from human complement-mediated injury, as manifested by an overall maintenance of myocardial function after challenge with human plasma.[37]

Recirculation of human whole blood in extracorporeal bypass circuits has been used extensively as a simulated model to study the activation of complement, leukocytes, and platelets in CPB.[15,16,28] The results of our extracorporeal circulation studies demonstrate that complete inhibition of the alternative complement cascade by anti-factor D MAb 166-32 suppresses activation of neutrophils and platelets, as well as inhibition of proinflammatory cytokine IL-8 production.

Methods

Design and Preparation of Extracorporeal Circuits

In the study whole blood was obtained from 5 healthy volunteers after approval by the Human Investigation Committee of Baylor College of Medicine. Informed consent was obtained from each participant. Whole blood from the same donor was used in both test and negative control circuits on the same day to avoid any variations in complement activity in donor blood.

Extracorporeal circuits were assembled by using a hollow-fiber pediatric membrane oxygenator with an integrated heat exchanger module (D 901 LILLPUT 1; DIDECO, Mirandola, Italy), a pediatric venous reservoir with an integrated cardiotomy filter (D 752 Venomidicard; DIDECO), a perfusion tubing set (Sorin Biomedical, Inc, Irvine, Calif), and a multiflow roller pump (Stöckert Instruments GmbH, Munich, Germany). Oxygenator and circuitry were primed with lactated Ringer's solution (Baxter Healthcare Corp, Deerfield, Ill). The prime fluid was warmed to 32°C with a cooler-heater (Sarns/3M Health Care, Ann Arbor, Mich) and circulated at 500 mL/min, and the sweep gas flow was maintained at 0.25 L/min with 100% oxygen. The sweep gas was changed to a mixture of oxygen (95%) and carbon dioxide (5%) after the blood was added to the circuit. The pH, P_{CO_2}, P_{O_2}, and perfusate temperature were continuously monitored throughout the recirculation period. Sodium bicarbonate was added as required to maintain pH in the range of 7.25 to 7.40.

Extracorporeal Circuit Operation and Blood Sampling

Blood (250 mL) was drawn over the course of 5 to 10 minutes into a transfer pack (Haemo-Pak; Chartermed, Inc, Lakewood, NJ) from healthy volunteers taking no medications. Before blood collection, porcine heparin (Elkins-Sinn, Cherry Hill, NJ) and anti-factor D MAb 166-32 (mouse immunoglobulin G_1) were added to a transfer pack at final blood concentrations of 5 U/mL and 18 μg/mL, respectively. This concentration of the antibody is equivalent to about 1.5 times the molar concentration of factor D in the blood. For the negative control, an isotype-matched irrelevant

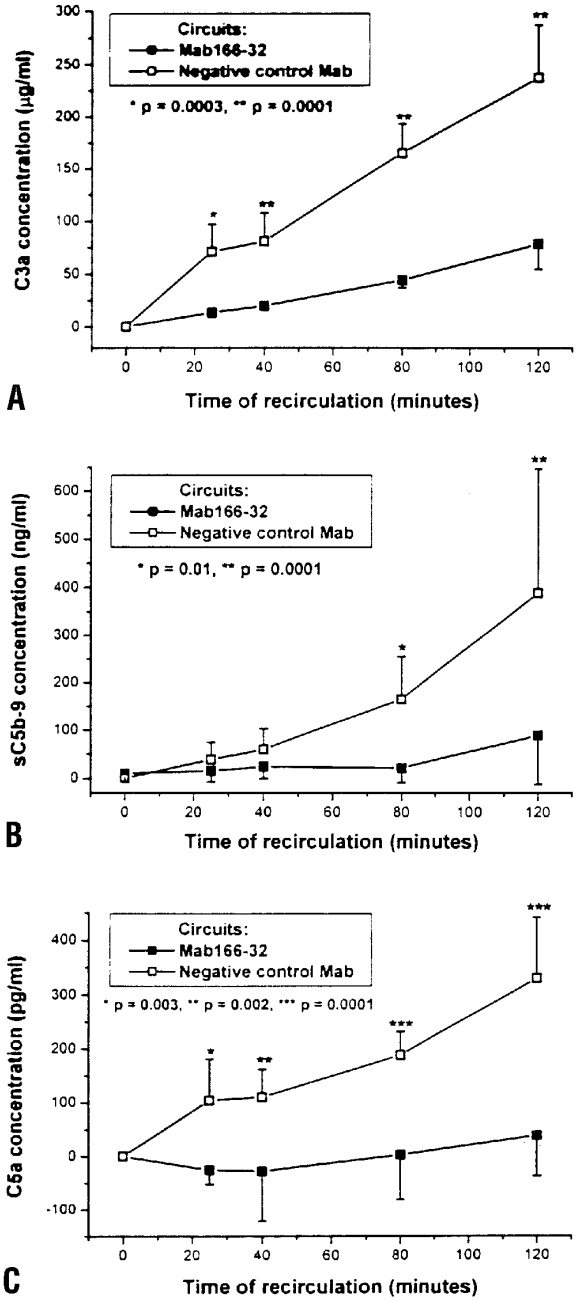

Figure 3. C3a, sC5b-9, and C5a production in the extracorporeal circuits treated with MAb 166-32 or the negative control MAb G3-519. C3a (A), sC5b-9 (B), and C5a (C) were measured by means of a quantitative ELISA. MAb 166-32 inhibited the production of C3a, sC5b-9, and C5a in the extracorporeal circuits compared with that in the MAb G3-519 circuits. Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

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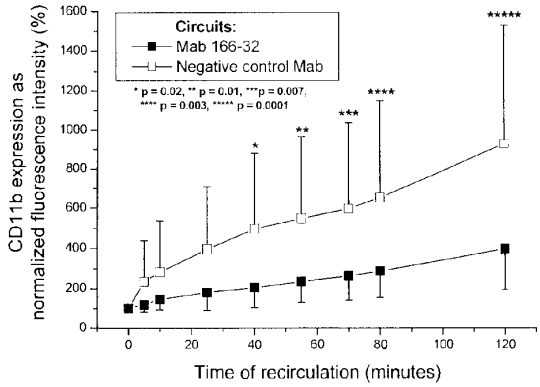

Figure 4. Inhibition of CD11b expression on PMNs in the extracorporeal circuits treated with MAb 166-32 compared with the circuits treated with the negative control MAb G3-519. The expression level of CD11b was measured by means of an immunofluorocytometric method and is expressed as the percentage of mean channel fluorescence intensity relative to the value at time 0 (100%). Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

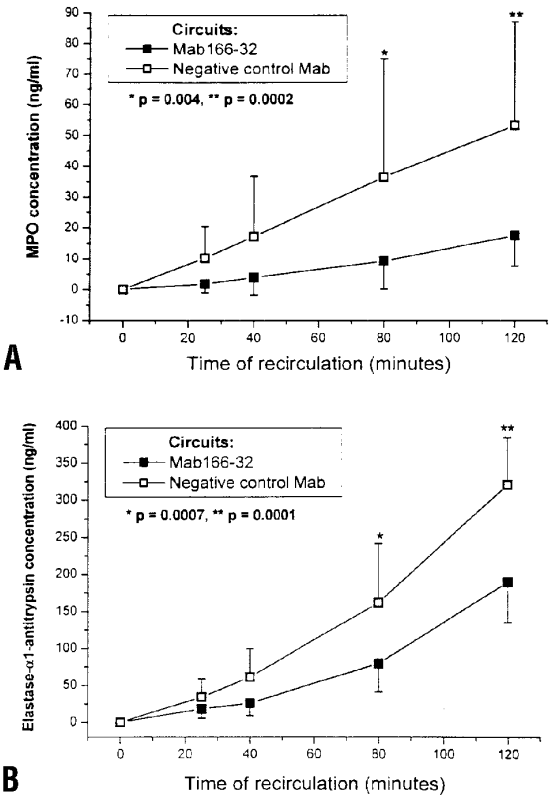

Figure 5. Inhibition of myeloperoxidase *(MPO)* and elastase release from neutrophils in the extracorporeal circuits treated with MAb 166-32 compared with that in the circuits treated with the negative control MAb G3-519. Myeloperoxidase (A) and elastase-α_1-antitrypsin inhibitor complex (B) were measured by a quantitative ELISA. Elastase released from activated neutrophils is quickly bound to α_1-antitrypsin inhibitor in the blood. Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

MAb (G3-519) was used at the same final concentration. MAb G3-519 (mouse immunoglobulin G_1) is specific to HIV-1 external envelope protein gp120. MAb G3-519 has previously been shown to have no effects on human complement activity.[37] The collected blood was then added to the reservoir through the prime port. Prime fluid was simultaneously withdrawn distal to the oxygenator outlet to yield a final circuit volume of 350 mL and a final hematocrit level of 25% to 28%. The pump flow rate was maintained at 500 mL/min, and the line pressure of the circuits was maintained at about 50 mm Hg throughout the entire experiment. Blood was recirculated with prime fluid, and complete mixing was accomplished within 3 minutes; a baseline sample was drawn and designated as time 0. In order to mimic the usual procedures of surgical operation under hypothermia, the circuit was then cooled to 27°C for 70 minutes, after which it was rewarmed to 37°C at 80 minutes. The temperature was maintained until 120 minutes, when the recirculation was terminated.

Blood samples were collected at 5, 10, 25, 40, 55, 70, 80, and 120 minutes during the recirculation. Plasma samples were prepared by immediate centrifugation at 2000*g* at 4°C. Aliquots of plasma were snap-frozen on dry ice and then stored at –80°C. The plasma samples were used for complement-mediated hemolytic assays and quantitation of neutrophil-specific myeloperoxidase and elastase, platelet thrombospondin, C5a, and IL-8. Aliquots of plasma for measurement of complement products C3a, C4d, sC5b-9, and Bb by means of enzyme-linked immunosorbent assay (ELISA) were immediately mixed with an equal volume of a specimen-stabilizing medium (Quidel, San Diego, Calif), snap-frozen on dry ice, and then stored at –80°C. Samples of whole blood were also collected for immunostaining of the activation cell-surface markers CD11b and CD62P on neutrophils and platelets, respectively. To prevent subsequent complement activation of the whole blood samples during the staining procedure, we added 10 μL of 1 mol/L ethylenediamine tetra-acetic acid to every milliliter of whole blood to give a final concentration of 10 mmol/L.

Complement-Mediated Hemolytic Assays

The alternative complement activity in the plasma samples from MAb 166-32–treated and MAb G3-519–treated circuits was measured with the use of rabbit red blood cells (RBCs). The plasma samples were treated with ethylene glycol-*bis*-(β-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid (EGTA) at a final concentration of 50 mmol/L to chelate Ca^{++} for inhibition of the classical complement. Thirty microliters of rabbit RBC suspension (1.7×10^8 cells/mL) in gelatin/veronal-buffered saline solution (GVB/Mg-EGTA) containing 2 mmol/L $MgCl_2$ and 50 mmol/L EGTA were added to 100 μL of human plasma (final plasma concentration,

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77%). After incubation at 37°C for 30 minutes, the supernatants were collected, and optical density (OD) was read at 405 nm by using an ELISA plate reader. This OD reading was designated as OD_A. One hundred microliters of each plasma sample was mixed with 30 μL of GVB/Mg-EGTA to give the plasma color background (OD_B). For 100% hemolysis, 100 μL of water was mixed with 30 μL of rabbit RBC suspension. This OD reading was designated as OD_{100}. The percentage of hemolysis was determined by the following formula:

$$\text{Percentage hemolysis} = 100 \times (OD_A - OD_B)/OD_{100}$$

Spontaneous lysis of RBCs was negligible.

For classical pathway hemolysis, chicken RBCs (5×10^7 cells/mL) in GVB^{++} containing 0.5 mmol/L $MgCl_2$ and 0.15 mmol/L $CaCl_2$ were sensitized with purified antichicken RBC immunoglobulins at 8 μg/mL (InterCell Technologies, Hopewell, NJ) for 15 minutes at 4°C. The cells were then washed with GVB^{++}. The plasma samples were not treated with EGTA. The remaining procedures were identical to those for alternative pathway hemolysis described above.

Assays of Complement Activation Products

Plasma samples from the extracorporeal circuits were tested for the levels of Bb, C4d, C3a, and sC5b-9. Bb is a specific marker for the activation of the alternative complement pathway, whereas C4d is for the classical complement pathway. These substances were measured by means of commercially available quantitative ELISA kits (Quidel) according to the manufacturer's manuals.

Assays for Expression of CD11b on Neutrophils and CD62P on Platelets

The activation of neutrophils and platelets was quantitated by measuring the levels of the cell-surface expression of CD11b and CD62P on neutrophils and platelets, respectively. For CD11b labeling of neutrophils, 100 μL of whole blood collected from the circuits was immediately incubated with 20 μL of phycoerythrin-anti-CD11b antibody (Becton Dickinson, San Jose, Calif) for 10 minutes at room temperature. Then 1.4 mL of FACS Lysing solution (Becton Dickinson) was added for 10 minutes at room temperature to lyse RBCs and to fix leukocytes. The cells were then washed in phosphate-buffered saline solution and then fixed in 1% paraformaldehyde before analysis with an EPIC-XL flow cytometer (Coulter Corp, Miami, Fla). For double labeling to concomitantly identify the neutrophil population, fluorescein isothiocyanate (FITC)-anti-CD15 antibody (Becton Dickinson) was added for incubation together with phycoerythrin-anti-CD11b antibody.

For CD62P labeling of platelets, 40 μL of whole blood collected from the circuits was incubated with 20 μL of phycoerythrin-anti-CD62P antibody (Becton Dickinson) for 10 minutes at room temperature. Then the mixture was treated with FACS Lysing solution as described above. The platelets were washed in phosphate-buffered saline solution, fixed in 1% paraformaldehyde, and then analyzed as described above. For double labeling to concomitantly identify the platelet population, FITC-anti-CD41 antibody (Coulter) was added for incubation together with phycoerythrin-anti-CD62P antibody.

For flow cytometric measurement, PMN and platelet populations were identified by live gating on the basis of forward-scatter versus side-scatter parameters and specific staining with FITC-anti-CD15 antibody and FITC-anti-CD41 antibody, respectively. The background staining was gated by means of isotype-matched labeled antibodies. The intensity of expression of CD11b and CD62P was represented by the mean fluorescence intensity.

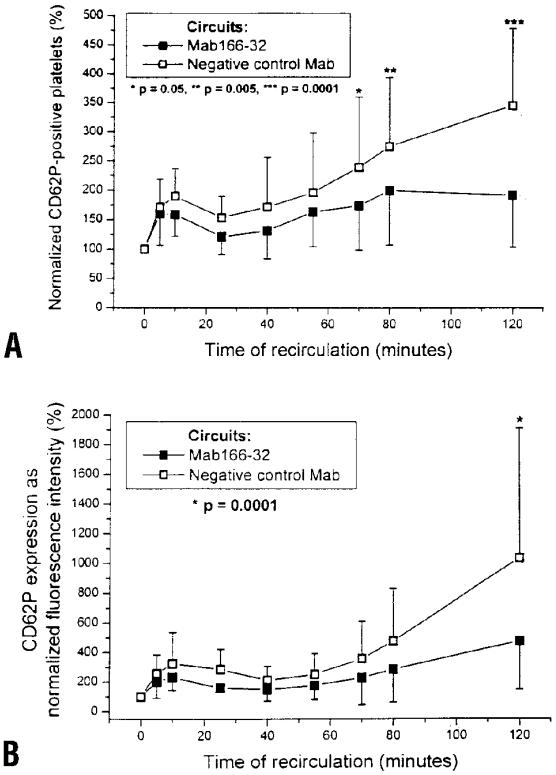

Figure 6. Inhibition of CD62P expression on platelets in the extracorporeal circuits treated with MAb 166-32 compared with the circuits treated with the negative control MAb G3-519. The percentages of $CD62P^+$ platelets (A) and the CD62P expression level in mean fluorescence intensity on platelets (B) are represented relative to the values at time 0 (100%). Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

Assay of C5a

C5a was measured quantitatively with an ELISA. Plasma samples were first treated with a precipitating reagent from a C5a radioimmunoassay kit (Amersham, Arlington Heights, Ill) to remove high-molecular-weight proteins, including C5. In the C5a ELISA purified rabbit anti-C5a antibodies (Calbiochem, La Jolla, Calif) were coated on wells of microtest plates to immobilize C5a in plasma samples. Purified sheep anti-human C5 antibodies (Biodesign, Kennebunk, Maine) were used to capture the immobilized C5a. The bound sheep antibodies were then detected by using horseradish peroxidase rabbit anti-sheep immunoglobulin G (Fc) antibodies (Jackson ImmunoResearch, West Grove, Pa). Peroxidase sub-

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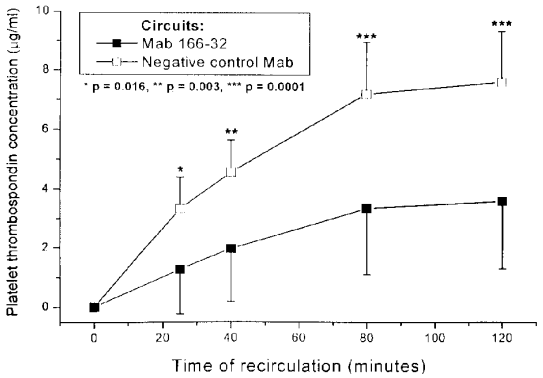

Figure 7. Inhibition of thrombospondin release from platelets in the extracorporeal circuits treated with MAb 166-32 compared with that in the circuits treated with the negative control MAb G3-519. Thrombospondin was measured by means of a quantitative ELISA. Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

strate solution was added for color development, and OD_{450} was measured by means of an ELISA plate reader. Purified C5a (Advanced Research Technologies, San Diego, Calif) was used as the standard for calibration.

Assay of Neutrophil-Specific Myeloperoxidase

Activation of neutrophils was also measured with an ELISA kit (R&D Systems, Inc, Minneapolis, Minn) to quantitate the amount of neutrophil-specific myeloperoxidase in the plasma samples from the extracorporeal circuits. The assays were performed according to the manufacturer's manual.

Assay of Elastase-α_1-Antitrypsin Inhibitor Complex

A quantitative ELISA was used to measure neutrophil-specific elastase-α_1-antitrypsin inhibitor complex, as described previously.[16] On activation, neutrophils release elastase, which is immediately complexed with α_1-antitrypsin inhibitor in the blood.[16] The assay used sheep anti-human neutrophil elastase polyclonal antibodies (Biodesign) to immobilize the complex and horseradish peroxidase–conjugated sheep anti-α_1-antitrypsin inhibitor polyclonal antibodies (Biodesign) for detection. As a calibration standard, elastase-α_1-antitrypsin inhibitor complex was made by mixing neutrophil-specific elastase (Elastin Products Company, Inc, Owensville, Mo) with α_1-antitrypsin inhibitor (Calbiochem).

Assay of Platelet Thrombospondin

A quantitative ELISA was used to measure platelet thrombospondin, which is an α-granule glycoprotein involved in platelet aggregation and is released on activation. In the assay thrombospondin was captured by using a pair of noncompetitive antithrombospondin MAbs, as described previously.[38] Monoclonal antithrombospondin antibody (clone P12, Coulter) was coated on wells of microtest plates to immobilize plasma thrombospondin. Biotinylated monoclonal antithrombospondin antibody (clone P10, Coulter) was used to capture immobilized thrombospondin. Purified thrombospondin (Calbiochem) was used as a calibration standard in the assay.

IL-8 ELISA

IL-8 was measured by using a quantitative ELISA kit (R&D Systems).

Statistical Analysis

The data from 5 paired circuits were statistically analyzed and represented as means ± SD. The data of hemolytic assays were analyzed with paired *t* tests. A 2-factor analysis of variance in a randomized block design was used to analyze the data from the other assays.

Results

Selective Inhibition of the Alternative Complement Activity by MAb 166-32

In the extracorporeal circulation study, MAb 166-32 was added to heparinized human whole blood at a final molar concentration of 1.5 times that of factor D in the blood. An isotype-matched negative control MAb, G3-519, was also studied at the same concentration in a parallel circuit by using whole blood from the same donor on the same day. The alternative and classical complement activities in the blood treated with the antibodies before recirculation were measured by means of hemolytic assays with rabbit RBCs and sensitized chicken RBCs, respectively. In the alternative pathway hemolytic assays, plasma from MAb G3-519–treated circuits at a final concentration of 77% induced lysis of rabbit RBCs by 65%, whereas no hemolysis was observed with plasma from MAb 166-32–treated circuits (Figure 1). In contrast, neither MAb 166-32 nor MAb G3-519 inhibited the lysis of sensitized chicken RBCs through the classical complement pathway (Figure 1). Therefore, MAb 166-32 is a selective and potent inhibitor of the alternative complement pathway.

Complement Activation in Extracorporeal Circuits

The selective inhibition of the alternative complement pathway by MAb 166-32 is in agreement with the complete inhibition of the production of Bb throughout the entire duration of recirculation (Figure 2, *A*). Bb is the enzymatic product of factor B on proteolysis by factor D, and therefore it is a specific marker for the activation of the alternative complement pathway. On the other hand, there was an increase in the plasma concentration of C4d in both circuits treated with either MAb 166-32 or MAb G3-519 (Figure 2, *B*). C4d is a specific marker for activation of the classical complement pathway.

The plasma concentrations of C3a, sC5b-9, and C5a in the extracorporeal circuits during recirculation were also measured. MAb 166-32 strongly inhibited the production of these substances (Figure 3, *A-C*). The formation of sC5b-9

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and C5a was completely inhibited during recirculation at hypothermic temperatures. At the end of 120 minutes of recirculation, MAb 166-32 inhibited the production of C3a, sC5b-9, and C5a by 67%, 77%, and 88%, respectively, compared with that of the negative control MAb G3-519. Because the alternative complement activity of the plasma in the test circuits was completely inhibited by MAb 166-32, the production of C3a, sC5b-9, and C5a in these circuits could be attributed to some activation of the classical pathway, as indicated by the elevation of plasma C4d levels (Figure 2, *B*).

Activation of Neutrophils in Extracorporeal Circuits

Activation of neutrophils as manifested by upregulation of surface expression of CD11b was measured by means of immunocytofluorometric analyses. The expression of CD11b on neutrophils in circuits treated with MAb 166-32 was significantly reduced compared with the negative control MAb G3-519 (Figure 4). At the end of 120 minutes of recirculation, MAb 166-32 inhibited CD11b expression on neutrophils by 57% compared with MAb G3-519.

The activation of neutrophils was also studied by measuring the levels of neutrophil-specific myeloperoxidase and elastase in plasma. The enzymes are stored in azurophilic primary granules of neutrophils and released on degranulation of activated neutrophils.[25-27] Consistent with the inhibition of neutrophil activation as manifested by the upregulation of CD11b expression (Figure 4), the release of both myeloperoxidase and elastase was also significantly inhibited by MAb 166-32 in the extracorporeal circuits compared with in the MAb G3-519–treated circuits (Figure 5, *A* and *B*). At the end of 120 minutes of recirculation, MAb 166-32 inhibited the release of myeloperoxidase and elastase by 67% and 41%, respectively, compared with MAb G3-519.

Activation of Platelets in Extracorporeal Circuits

The effects of MAb 166-32 on platelet activation during recirculation in extracorporeal circuits were examined. The number of $CD62P^+$ platelets and the level of CD62P expression were significantly reduced in MAb 166-32–treated circuits compared with in MAb G3-519–treated circuits (Figure 6, *A* and *B*). At the end of 120 minutes of recirculation, the number of $CD62^+$ platelets and the level of CD62 expression were reduced by 45% and 54%, respectively, in MAb 166-32–treated circuits compared with in MAb G3-519–treated circuits. These results corroborate with the inhibition of thrombospondin release from activated platelets by MAb 166-32 treatment by 53% at the end of 120 minutes of recirculation compared with MAb G3-519 (Figure 7).

Production of IL-8 in Extracorporeal Circuits

To further examine the effects of complement inhibition on other inflammatory responses in whole blood during recirculation, we examined the production of the proinflammatory cytokine IL-8 in the test and control extracorporeal circuits. IL-8 is produced predominantly by activated neutrophils, monocytes-macrophages, and T cells. During the recirculation at hypothermic temperatures, the plasma levels of IL-8 did not increase, even in MAb G3-519–treated circuits. However, the plasma levels of IL-8 increased dramatically when the circuits were warmed to 37°C. At the end of 120 minutes of recirculation, IL-8 production in MAb 166-32–treated circuits was significantly reduced by 73% compared with MAb G3-519–treated circuits (Figure 8).

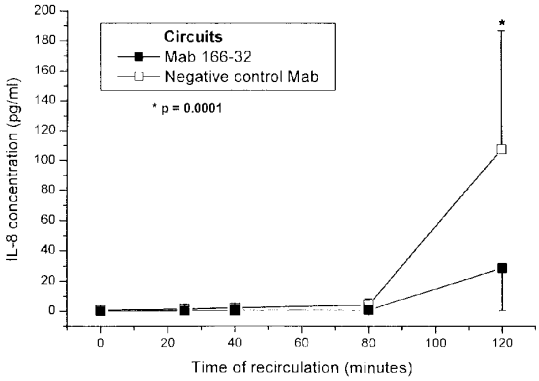

Figure 8. Inhibition of IL-8 production in the extracorporeal circuits treated with MAb 166-32 compared with that in the circuits treated with the negative control MAb G3-519. IL-8 was measured by a quantitative ELISA. Values are given as means ± SD from 5 paired experiments of the test and control circuits. *P* values were determined by 2-factor analysis of variance.

Discussion

The complement cascade is activated predominantly through the alternative or the classical pathway.[12] Activation of the alternative complement pathway is induced through the direct activation of C3, whereas the classical complement pathway is activated through the initial activation of C1. Recent studies show that complement can also be activated through the lectin pathway, which involves the initial binding of mannose-binding lectin and the subsequent activation of C2 and C4, which are common to the classical complement pathway.[39,40] Interestingly, accumulating evidence indicates that the alternative complement pathway participates in the amplification of the activity of both the classical complement and the lectin pathways as a result of C3b deposition, which triggers the formation of the alternative C3/C5 convertases.[40,41] Therefore, the alternative complement pathway may play a broad role in inflammation resulting from complement activation.

In CPB circuits the alternative complement pathway plays a predominant role in complement activation, result-

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ing from the interaction of blood with the artificial surfaces of CPB circuits.[1,6,11] However, natural human antibodies against dialysis membranes have been described, and it is possible that natural antibodies against components of the CPB circuits or membrane could be responsible for the C4d production in our study.[42] Interestingly, the alternative complement pathway was shown to play a crucial role in the amplification of the classical complement pathway once it was initiated.[42] In addition, activation of the classical complement pathway in CPB circuits could also be triggered by means of contact activation of the intrinsic coagulation pathway.[10,43,44] As a result, factor XIIa, factor XIa, and kallikrein bind C1 esterase inhibitor, an endogenous inhibitor of activated C1. When the plasma concentration of C1 esterase inhibitor is diminished, C1 becomes more susceptible to activation, and thus the classical complement pathway is augmented. Inasmuch as the production of C5a and sC5b-9 was substantially inhibited compared with that of C3a in our study, the moderate activation of the classical pathway as indicated by the formation of C4d during hypothermic recirculation does not seem to effectively activate C5. Similarly, although protamine neutralization of heparin after CPB causes activation of the classical complement pathway,[6,45] the activation of C5 by protamine-heparin complexes is found to be ineffective.[6,8] Using anti-factor D MAb 166-32 to selectively inhibit the alternative complement pathway, we confirm the previous findings that the alternative complement pathway is predominantly activated in CPB circuits.[1,6,8]

The results from our study show that anti-factor D MAb 166-32 is a potent and specific inhibitor of the alternative complement pathway. At a concentration (18 μg/mL) equivalent to 1.5 times the molar concentration of factor D in human blood, MAb 166-32 completely inhibits the alternative complement activity, as evidenced by the complete blockade of Bb formation in the CPB circuits. Inhibition of the alternative complement pathway by anti-factor D MAb 166-32 suppresses the production of C3a, sC5b-9, and C5a. These substances are known to activate neutrophils, monocytes, platelets, mast cells, and endothelial cells.[13-16,28,29] Reduction of CD11 (α-integrin) expression on neutrophils prevents their binding to platelets and endothelial cells through the interaction with CD62P on these cells.[46,47] Sequestration of activated neutrophils on endothelial cells can result in tissue inflammation and injury.[24,29,30] It has been shown that CD11b expression on monocytes is increased by C3a but not C5a and sC5b-9 in CPB circuits.[15,16] Therefore, the reduction of C3a production by MAb 166-32 in our study could be important for controlling monocyte adhesion. Although the exact roles of activated monocytes in the inflammatory response of CPB have yet to be delineated, it is likely that proinflammatory cytokine release and tissue factor–mediated thrombosis are involved.[13,48] Because CR3 (Mac-1; CD11/CD18) on leukocytes can bind to iC3b on complement-activated surfaces in CPB circuits,[49] this could contribute to the depletion of leukocytes commonly observed in patients undergoing CPB.[15,44] Inhibition of CD11b upregulation by MAb 166-32 in our study could prevent the loss of leukocytes during extracorporeal circulation.

The results from our study also show that inhibition of the alternative complement pathway by anti-factor D MAb 166-32 suppresses the degranulation of neutrophils, as indicated by the reduced release of myelopoxidase and elastase. Myeloperoxidase is responsible for production of hypochlorous acid, a potent oxidant that could cause tissue damage, whereas elastase could mediate tissue injury as a result of hydrolysis of elastin in extracellular matrices.[25-27] The incomplete inhibition of neutrophil degranulation by anti-factor D MAb 166-32 in this study might be attributed to contact activation, which results in the production of kallikrein, a mediator that is known to activate neutrophils to release elastase.[50] Similarly, platelets could be activated by thrombin as a result of initiation of the intrinsic coagulation pathway after contact activation.[51]

IL-8 is closely associated with inflammatory diseases and CPB.[19-22] Our results show that anti-factor D MAb reduces the release of IL-8. IL-8 causes chemotaxis of neutrophils, T cells, and basophils; degranulation of neutrophils; and adhesion of neutrophils to endothelial cells.[30] Therefore, the reduction of IL-8 production could reduce multiple inflammatory responses observed in CPB. Delayed increase of plasma IL-8 in our extracorporeal circulation study is consistent with the observation in operations involving CPB.[19,21,22]

One limitation of the simulated extracorporeal circulation model of CPB used in this study is the inability to study the inflammatory responses of endothelial cells caused by complement activation. In this regard, both C5a and C5b-9 were shown to induce surface expression of P-selectin on endothelial cells.[29]

Several complement inhibitors are being studied for potential applications in CPB. They include a recombinant soluble complement receptor 1 (sCR1),[52] a humanized single-chain anti-C5 antibody (h5G1.1-scFv),[53] a recombinant fusion hybrid (CAB-2) of human membrane cofactor protein and human decay accelerating factor,[16] and a 13-residue C3-binding cyclic peptide (Compstatin).[54] sCR1 and CAB-2 inhibit the classical and alternative complement pathways at the steps of C3 and C5 activation. Compstatin inhibits both complement pathways at the step of C3 activation, whereas h5G1.1-scFv does so only at the step of C5 activation. The results of our study strongly indicate that the alternative complement activation plays an important role in inducing a plethora of cellular and humoral inflammatory responses in CPB. Anti-factor D MAb 166-32 is effective in

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inhibiting the activation of C3 and C5 and the other inflammatory responses in extracorporeal circulation. Therefore, complete inhibition of the alternative complement pathway by targeting factor D could be useful to reduce systemic inflammation in patients undergoing CPB.

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Pathogenic Roles of B Cells in Human Autoimmunity: Insights from the Clinic

Review

Flavius Martin[1,*] and Andrew C. Chan[1,2,*]
[1]Department of Immunology
[2]Department of Antibody Engineering
Genentech, Inc.
One DNA Way, MS-34
South San Francisco, California 94080

The pathogenic roles of B cells in human autoimmune diseases involve a multitude of mechanistic pathways and include the well-established contributions of autoantibodies and immune complexes that induce local inflammatory reactions and tissue destruction. Recent results using several novel B cell-directed therapies have provided new insights into additional roles of B cells in human autoimmunity. In this review, we will highlight some of these studies and discuss how clinical insights parallel murine models of normal immunity and autoimmunity.

Treatment of autoimmune disorders has begun a transformation from an armamentarium of nonspecific therapies that affect a wide range of cells and signaling pathways to targeted therapies that modulate specific cytokines (e.g., tumor necrosis factor and IL-1), cell types (e.g., $CD3^+$ T cells and $CD20^+$ B cells), or inhibit a unique signaling pathway (Kamradt and Mitchison, 2001; Davidson and Diamond, 2001; Keystone, 2003; Gottlieb, 2003; Shanahan et al., 2003). The advent of targeted therapies in the treatment of human autoimmune diseases has not only significantly improved patients' lives and changed clinical therapeutic paradigms but has also led a renaissance in understanding pathogenesis of human disorders (Anasetti et al., 1992; Weinblatt et al., 1999; Lipsky et al., 2000; Pisetsky, 2000b; Cohen et al., 2002; Kremer et al., 2003; Keystone et al., 2003; Edwards et al., 2004). Our insights into the pathogenic roles of B cell dysfunction in human autoimmune diseases circa 2004 remain limited. While prophylactic or interventional preclinical studies in rodent models of human diseases are enlightening, many of these genetically homogeneous models do not translate well into the heterogeneous nature of human biology and diseases. Hence, a challenge to drug discovery as well as human immunology is to understand how probing B cell biology in human autoimmunity translates back to these preclinical models of human disease and then applying this new knowledge to advance patient care. In this discussion, we will review how therapies in human autoimmune diseases have provided insights into our expanding understanding of B cell functions in human autoimmune disorders (Figure 1).

The scientific routes of B cell modulation began with the elegant surgical approaches in birds and underscored the importance of the Bursa of Fabricius in B cell ontogeny (Cooper et al., 1965). This was followed by pioneering work using anti-immunoglobulin (Ig) reagents in which pre- and perinatal treatment of mice with anti-Ig polyclonal reagents successfully depleted surface positive B lineage cells (Cooper et al., 1980). Such a strategy was not applicable to adult animals that have high levels of circulating Igs that function as a sink for the depleting anti-Ig reagents. Attempts to translate these observations into clinical use for B cell lymphomas focused initially around the idea of anti-idiotype (Id) antibodies passively delivered or induced through active immunization (anti-Id vaccines) (McCarthy et al., 2003). Although this approach seemed ideal from a scientific perspective, the preexisting suppressed immune systems of cancer patients, phenomenon of tumor escape, as well as manufacturing related factors have delayed the translation of these therapies to clinical practice. The alternative approach of using antibodies directed against pan-B cell targets was faster to reach fruition through the development of antibodies targeting the CD20, CD22, and CD52 surface molecules (Treon and Anderson, 2000; Juweid, 2003; Cersosimo, 2003; Robak, 2004). All these approaches rely on the ability of passively infused antibodies to deplete antigen-bearing cells. This depletion is temporary and ceases through the continuous repopulation of the B lineage from precursors residing within primary hematopoietic organs.

In contrast to the B cell malignancies, non-Hodgkin's lymphoma, and chronic lymphocytic leukemia, in which one or more proliferating B cell clones can be identified at a cellular and molecular level, the identity and specificity of the pathogenic B cell clones in autoimmune diseases are rarely known. In a subset of lymphoma and leukemia patients, the malignant B cell clones produce low-affinity paraproteins (antibodies) that manifest autoimmune paraneoplastic syndromes. These paraproteins bind a variety of self-antigens, including those expressed on red blood cells (RBCs) that result in autoimmune hemolytic anemia (e.g., IgM cold agglutinin disease), or C1 esterase complement inhibitor that manifests clinically with angioedema (Silberstein, 1994; Pascual et al., 1997; Ward, 2001). Treatment of the underlying lymphoma or leukemia and eradication of the malignant B cell clone improves the associated clinical autoimmune manifestations. For most autoimmune diseases, however, the mechanistic relationship between pathogenic B cells, their products (e.g., autoantibodies), and clinical manifestations remains elusive. Nonetheless, the emerging findings suggest that B lineage depletion or modulation might be beneficial and provides a basis to further explore the role of B lineage function in human biology.

Autoantibodies—Diagnosis and Pathogenesis

Autoantibodies have served historically as the sine qua non to indicate a role for B cells in autoimmune diseases. They provide diagnostic and prognostic criteria, play a requisite role in disease pathogenesis, and serve as surrogate markers for disease activity.

The first category, while not mutually exclusive with the others, represents a large proportion of autoantibodies and typically is not thought to play a role in disease

*Correspondence: flavius@gene.com (F.M.); acc@gene.com (A.C.C.)

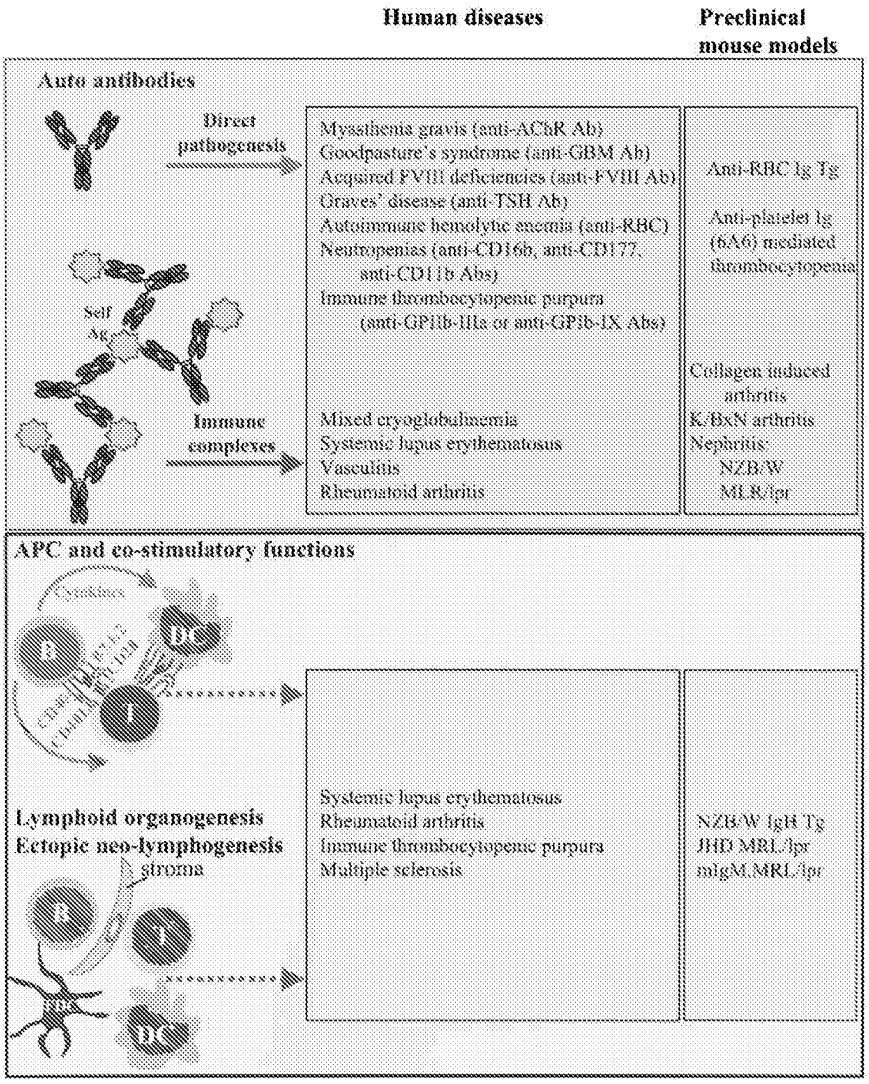

Figure 1. Effector Mechanisms of Human and Mouse B Cell Autoimmunity

"Classic" antibody-dependent mechanisms are responsible either directly or through immune complexes for the pathogenesis of specific autoimmune diseases. Several other mechanisms, including antigen presentation (APC), costimulatory functions (surface molecules and secreted cytokines), and the ability to support de novo lymphoid tissue organization (neolymphogenesis) may explain other parts of B cell involvement in complex autoimmune conditions.

AchR, acetylcholine receptor; GMB, glomerular basement membrane; FVIII, factor VIII; TSH, thyroid-stimulating hormone; RBC, red blood cells; GPIIb-IIIa, glycoprotein IIb-IIIa; GPIb-IX, glycoprotein Ib-IX; Ag, antigen; B, B cell; T, T cell; FDC, follicular dendritic cell; DC, dendritic cell.

pathogenesis. However, the latter may merely reflect our lack of understanding of antigen specificity and its role in disease pathogenesis. Nonetheless, many antibodies provide highly sensitive and/or specific diagnostic criteria for the presence of a specific clinical syndrome. For example, the presence of anti-nuclear antibodies (ANA) serves as an extremely sensitive diagnostic test for systemic lupus erythematosus (SLE). This autoimmune disorder is characterized by T, B, and myeloid cell dysregulation that gives rise to immune complex (IC)-mediated deposition and inflammatory damage in a variety of organs, including skin, lung, kidney, blood vessels, and the nervous system (Pisetsky, 2000a). However, this serologic marker is observed in a variety of other autoimmune disorders, and the level of serum ANAs does not correlate with the severity of clinical disease. Patients can have persistently high ANA titers despite the absence of clinical disease. Conversely, patients experience disease flares without any alterations in ANA levels. Other types of autoantibodies in SLE patients can also be used to aid diagnosis and prognostication of patient subsets—the presence of

anti-Sm antibodies is highly specific for SLE diagnosis, anti-RNP antibodies identify a subset of patients with a different clinical course of mixed connective tissue disease, anti-phospholipid antibodies are associated with an increased risk of thrombotic events, anti-ribosomal P antibodies identify patients with increased risk of central nervous system disease, and anti-Ro (SSA) antibodies are causally linked with an increased risk for the development of complete heart block (CHB) in the newborns of SLE patients (Ruiz-Irastorza et al., 2002; Reichlin, 2003; Buyon and Clancy, 2003). In the case of anti-Ro antibodies, only 50% of women have symptomatic SLE or Sjogren's syndrome at the time their babies are born with CHB. Yet, a large proportion of these asymptomatic women ultimately develop clinical SLE and Sjogren's disease over the ensuing decade (Brucato et al., 2002).

Aside from rheumatologic disorders, examples of pathogenic autoantibodies include the anti-acetylcholine receptor (AChR) and anti-glomerular basement membrane (GBM) antibodies in myasthenia gravis and Goodpasture's syndrome, respectively. Anti-AChR antibodies alter AChR organization on the postsynaptic neuronal plate and functionally block acetylcholine neurotransmission to induce muscle paralysis, in particular impairment of diaphragmatic function, with progression to respiratory failure and death (Vincent, 2002). The histological demonstration of these complement fixing antibodies on myelinated fibers from affected patients and ability of plasma exchange and intravenous immunoglobulin (IVIG) to improve clinical course together support a pathogenic role for anti-AChR antibodies in MG (Richman and Agius, 2003). Binding of anti-GBM antibodies to the $\alpha 3$ chain of type IV collagen in the lung and kidneys results in pulmonary hemorrhage and glomerulonephritis, respectively, the latter of which can progress to renal failure. Suppression and removal of these pathogenic antibodies with immunosuppressive agents and plasma exchange guide the therapeutic principles of anti-GBM disease (Hudson et al., 2003). Hence, both of these pathogenic antibodies support a definitive role for B lineage cells in human disease.

Immune Complexes, Complement, and Fc Receptors in Disease Pathogenesis

The contributions of antibodies to disease are not solely dependent upon their direct binding to end-organ tissue antigens but also through indirect mechanisms, including IC formation, IC deposition, complement activation, and FcR activation. These complement-activating ICs are found in many autoimmune disorders and deposit in a variety of tissues (e.g., kidney, lung, and blood vessels) to cause end-organ damage (e.g., kidney failure, pulmonary hemorrhage, and vasculitis, respectively). A classical example of an IC-mediated disease is the Hepatitis C-associated syndrome of mixed cryoglobulinemia, in which complement activating IgG and IgM ICs (containing Hepatitis C antigen) are deposited in the skin, kidney, and peripheral nerves to induce cutaneous purpuric lesions, glomerulonephritis, and mononeuritis multiplex, respectively (Trendelenburg and Schifferli, 2003). Treatment is dependent upon the extent of involvement but traditionally geared toward decreasing cryoglobulin production through the use of immunosuppressive therapies and, in severe cases, removal of the pathogenic complexes through plasma exchange.

A role for ICs in autoimmune disorders is also supported by the genetic association of Fc receptor (FcR) polymorphisms and human disease. FcRs for IgG include the high-affinity FcγRI (CD64) receptors and the low-affinity FcγRII (CD32) and FcγRIII (CD16) receptors. Genetic polymorphisms for both low-affinity IgG receptors have been described (Ravetch and Bolland, 2001; Binstadt et al., 2003). The major FcγRIIA polymorphism resides at amino acid 131. FcγRIIA(His131) confers higher affinity for IC binding than FcγRIIA(Arg131). His131/His131 phagocytes are more efficient in IC phagocytosis and clearance than cells bearing the Arg131/Arg131 low-affinity receptor allelic genotype. The major FcγRIIIA polymorphism resides at amino acid 158. Val158 confers high-affinity binding while Phe158 confers low-affinity binding. Patients expressing two copies of FcγRIIA(Arg131/Arg131) or IIIA (Phe158/Phe158) receptors have been reported in some studies but not in others to exhibit increased association with a number of autoimmune diseases, including immune-mediated thrombocytopenia (ITP), SLE, and multiple sclerosis (MS). These polymorphic differences may result not only in prolonged IC circulation, deposition, and tissue damage but may enhance inflammatory and proliferative responses of FcR-bearing cells in disease.

The ability of ICs to activate the complement cascade and liberate proinflammatory complement degradation components that enhance IC clearance, augment phagocyte activation, and modulate cellular and humoral immunity provide additional effector mechanisms by which B cell products can contribute to disease (Holers, 2003; Walport, 2001a, 2001b). In preclinical models, inhibition of complement activation, using soluble forms of complement receptors or antibodies that bind complement degradation products, have demonstrated efficacy in preclinical models of ischemia-induced injury, lupus, and collagen-induced arthritis (Weisman et al., 1990; Couser et al., 1995; Wang et al., 1995, 1996). Translation of some of these principles to the clinics are in progress and will provide us with a greater understanding of these effector pathways in autoimmune disease. A phase II study of the anti-C5 inhibitor antibody eculizumab (5G1.1, Alexion Pharmaceuticals, Cheshire, CT) that prevents the generation of the C5a anaphylatoxin has demonstrated short-term safety and tolerability in patients with rheumatoid arthritis (RA) and clinical benefit compared to control patients (Tesser et al., 2001).

The therapeutic efficacy of IVIG in a limited subset of autoimmune diseases, including ITP and MG, also suggests roles for the contribution of antibodies, complement, and FcRs in B cell-mediated autoimmunity. ITP is a clinical syndrome manifested by immune-mediated destruction of platelets (Beardsley, 2002). These patients demonstrate high levels of autoantibodies directed against platelet surface glycoproteins that are associated with accelerated platelet destruction (McMillan et al., 2001; McMillan, 2003). IVIG is manufactured from pooled human plasma, and its therapeutic effects in ITP involve a multitude of mechanisms that include Fc blockade to decrease clearance of antibody-laden platelets, inhibition of complement deposition, and more

recently evidence for engagement of inhibitory FcγRIIB receptors. In a murine model of autoantibody-induced ITP, the therapeutic effect of IVIG was dependent upon the induced expression of the inhibitory FcγRIIB receptor on splenic macrophages that, in turn, inhibits Fc-mediated opsonization of antibody-bound platelets (Samuelsson et al., 2001). Similarly, the protective effects of IVIG in the K/BxN arthritis model are dependent on the ability of CSF-1-dependent macrophages to upregulate FcγRIIb expression on CSF-1-independent "effector" macrophages (Bruhns et al., 2003). Hence, IVIG may function, in part, by inhibiting effector responses through this inhibitory receptor. Independent of mechanism, the limited therapeutic benefits of IVIG in specific autoimmune disorders support a pathogenic role for antibodies and ICs in these diseases.

The therapeutic application of plasma exchange has provided additional support for the role of antibodies and ICs to implicate B lineage cells in disease (Saydain et al., 2002). Therapeutic apheresis is a technique by which plasma and cellular components are separated and autoantibodies, ICs, or toxins are removed from the plasma. This technique has been modified to include protein A immunoadsorption to specifically extract pathogenic autoantibodies and ICs. While plasma exchange has been used in a wide number of autoimmune diseases, its therapeutic application is accepted as standard of care in only a handful of autoimmune diseases, including anti-GBM disease and MG, and adjunctive therapy in cryoglobulinemia and ITP. Nonetheless, modulation of circulating ICs and pathogenic antibodies by simple removal has proved effective in some of these B lineage-mediated diseases.

B Cell Depletion

The development of therapeutics that specifically target B cells during the past decade has provided an entrée to better translate preclinical observations into human biology and disease. These therapeutics have been led by the use of rituximab (Rituxan®, Genentech, Inc., South San Francisco, CA and Biogen-IDEC, Cambridge, MA; Mabthera®, F.Hoffman-LaRoche, Ltd., Basel, Switzerland), a chimeric monoclonal antibody directed against the CD20 molecule. CD20 is a transmembrane glycoprotein expressed exclusively on normal and malignant B cells. Its expression is regulated during B cell development emerging in late pre-B cells and extinguished on plasma cells. Given the prominent historical role of autoantibodies, the absence of CD20 on plasma cells that presumably are responsible for producing autoantibodies raised fundamental skepticism in applying this therapy to autoimmune diseases. Yet, the efficacious use of Rituxan in a number of autoimmune disorders has brought into question some of the preconceptions of the biology of B lineage cells in autoimmune disorders. Ideally, one would design a B cell-depleting therapy for autoimmunity to meet several criteria: (1) deplete all or most pathogenic B lineage cells or their precursors (assuming that pathogenic cells and their products have a reasonably short lifespan), (2) pathogenic B cell repopulation should not occur or should be significantly delayed upon repletion from early bone marrow progenitors, (3) interrupt or influence non-B cell effector pathogenic pathways that are dependent or downstream of B cell-initiated processes. To date, clinical results exist to support the first two criteria while the third awaits more formal scientific validation.

Clinical data for Rituxan in autoimmune diseases is emerging from a variety of double-blinded placebo-controlled trials though a large amount of data has already emerged from smaller Phase I/II studies and case series reports (Edwards et al., 2002; Silverman and Weisman, 2003). The use of Rituxan in ITP led its entrée into the realm of autoimmune diseases. In four case series reports, 33%–54% of ITP patients, who had failed standard of care corticosteroid and splenectomy therapies, experienced partial or complete responses to Rituxan, in most cases in the absence of concomitant corticosteroids (Saleh et al., 2000; Stasi et al., 2001; Zaja et al., 2003; Cooper et al., 2004). Interestingly, two patterns of clinical responses emerged. Early responders experienced immediate increases in platelet counts after the first or second antibody infusion that were followed by a continued rise until a peak count was achieved between weeks 6 and 10. In contrast, late responders had minimal immediate effects. Rather, they had a delayed effect in which peak counts were achieved within the 6 to 8 week period. These distinguishing patterns suggest that Rituxan may operate through at least two distinct mechanisms in ITP. In the first, the effects of Rituxan are too rapid to be accounted for by depletion of circulating antiplatelet antibodies but potentially could be expanded through Fc receptor-mediated functions. In the latter phase, the sustained effects may function through alterations in anti-platelet antibodies and/or interruption of T-B cell cooperation or other B cell-dependent events in disease pathogenesis. To date, these small clinical studies reveal no direct correlation of anti-platelet antibody levels with clinical responses and raise the likelihood of additional mechanisms by which B lineage cell depletion modulates ITP (see below).

The use of Rituxan in autoimmune disorders is most advanced in the treatment of patients with rheumatoid arthritis (RA). This disease involves a multicellular inflammatory response involving infiltration of lymphocytes and granulocytes, proliferation of synovial fibroblast and macrophages, and neovascularization of the lining surrounding joints. This proliferative process not only induces swelling, erythema, and pain of multiple joints but progress to destruction and loss of bone density and architecture. The diagnosis of RA is aided typically by the presence of low-affinity IgM rheumatoid factor (RF) autoantibodies directed against the Fc portion of IgG. In a multicenter randomized double blind controlled study of 161 patients, those treated with Rituxan and a short course of corticosteroids in conjunction with methotrexate or cyclophosphamide achieved greater clinical responses than patients treated with corticosteroids and methotrexate alone (Shaw et al., 2003; Edwards et al., 2004). Analysis of serologic changes in a smaller series of 22 RA patients demonstrated a 60% decrease in IgM RF and 75% decrease in anti-cyclic citrullinated peptide (CCP) antibodies in Rituxan responders (Cambridge et al., 2003). Despite the longer half-life of IgG, when compared to IgM, IgG RF decreased more rapidly and to a greater extent (80%) than IgM RFs (60%) following Rituxan therapy. In contrast, no

change in anti-pneumococcal capsular polysaccharide IgG antibodies and a 23% decrease in anti-tetanus toxoid IgG antibodies were detected 3 months following treatment. Similar serologic effects have been observed in two additional studies with Rituxan treatment (De Vita et al., 2002; Tuscano, 2002). This preferential decrease in autoantibodies suggests that autoreactive B cells and their corresponding plasma cells may have a shorter lifespan while non-self-reactive plasma cells are relatively more resistant to Rituxan and have a longer lifespan.

Since RF is not thought to play a direct pathogenic role in rheumatoid arthritis, as transfer of RF-positive serum in the *skg* model of RA is not sufficient to confer disease (Sakaguchi et al., 2003), the therapeutic efficacy of Rituxan reported in these studies identifies B lineage cells as playing a key, but undefined, pathogenic role in RA. B cells exist as lymphoid aggregates within the synovium of RA patients and may function as APCs to provide important costimulatory signals in promoting effector T cell expansion (Weyand and Goronzy, 2003). Addition of antibodies that block T and B cell cooperation results in loss of these T-B cell aggregates (Takemura et al., 2001). Hence, B cell depletion may remove a critical component of T-B cell cooperation required for disease. While RF alone is not sufficient to induce disease, RF-containing ICs may promote synoviocytes to induce local release of inflammatory factors and exacerbate the inflammatory cascade. Conversely, decreases in RF and other autoantibodies may attenuate these local inflammatory ICs and result in clinical improvement. Additional clinical studies of synovial B cells prior to and following Ritxuan therapy will be revealing in dissecting the specific mechanistic roles of systemic and local (e.g., synovial) B cells in RA and the effects of Rituxan on RA pathogenesis.

Longer term follow-up analysis of a handful of Rituxan-treated RA patients have been reported and reveal that most undergo clinical relapse. In the study reported by Cambridge and colleagues, 13 of 15 responders relapsed within 17 months following B cell repletion. In all but one patient, return of autoantibodies preceded relapse. Return of autoreactive antibodies, through their corresponding plasma cells and precursors, may occur through a stochastic recapitulation of pathogenic events involving a new set of naive B cells freshly generated from the bone marrow. Supporting this scenario are data on subset composition for repopulating blood cells as well as their VH gene repertoire assayed by single cell PCR (Rouziere et al., 2003). Peripheral B cells from patients with moderate to severe RA have a large component of isotype-switched memory cells with heavily somatically mutated VH genes. During and immediately following B cell repletion, the repertoire is dominated by phenotypically naive cells with germline VH regions. Preceding reemergence of autoantibodies and clinical relapse, the peripheral B cell repertoire begins reaccumulating mutations and slowly converges to resemble the predepletion repertoire. Together, these early studies suggest that pathogenic B cell clones and their progeny can be modulated by Rituxan therapy and that autoreactive and allogeneic responses likely have distinct kinetics of longevity and biology.

Recent studies of four patients with circulatory inhibitors against Factor VIII, an important component of the coagulation cascade, also demonstrate differences between self and non-self antibodies. These patients have circulating FVIII inhibitors (anti-FVIII autoantibodies), despite immunosuppression with corticosteroids and chemotherapeutic agents, which functionally compromise their coagulation cascade, that result in bleeding complications requiring RBC transfusions. Treatment with Rituxan resulted in a rapid decrease in their acquired FVIII inhibitor and return of their endogenous FVIII levels (Wiestner et al., 2002). Interestingly, one of these four patients had mild hemophilia A and received recombinant human FVIII (rFVIII) perioperatively, which appears to have triggered an increase in FVIII inhibitor activity and further compromised his already low FVIIIc level. Administration of Rituxan in this patient resulted in rapid resolution of the autoantibody (against endogenous FVIII) whereas the alloantibody (anti-rFVIII) response persisted for months. Hence, similar to the fate of serum RF, the biologic effects of these autoreactive B cells appear to be far shorter lived than non-self-reactive B cells.

In addition to ITP, RA, and FVIII deficiency, therapeutic effects of Rituxan have been reported in small case series of patients with pemfigus vulgaris (anti-desmoglein-3 antibodies) and pemfigus foliaceus (anti-desmoglein-1 antibodies) (Salopek et al., 2002; Goebeler et al., 2003; Dupuy et al., 2004). In addition to clinical benefit, pathogenic antibody titers decrease, but do not disappear, following Rituxan administration and return at relapse. In at least one patient, clinical relapse and autoantibody increase was documented in the complete absence of peripheral B cells, suggesting a complex relationship between B cells, autoantibodies, and clinical effect. Patients with MG have similarly demonstrated decreases in anti-AChR antibodies, which is accompanied by a slow clinical improvement (Wylam et al., 2003). Finally, patients with IgM-associated polyneuropathies with anti-MAG (myelin-associated glycoprotein) autoantibodies have also been reported to have decreased anti-MAG titers and clinical improvement following Rituxan administration (Levine and Pestronk, 1999; Pestronk et al., 2003). In contrast, two patients with anti-ganglioside IgG antibodies did not improve even up to 1 year following depletion (Rojas-Garcia et al., 2003). This dichotomy of responsiveness does not simply reflect differences between IgM and IgG autoantibodies since other IgG autoantibodies are modulated by Rituxan therapy.

The effects of Rituxan on autoreactive and non-self-reactive antibodies have only recently been studied in SLE patients. Three open label studies have been reported (Leandro et al., 2002; Anolik et al., 2003; Eisenberg, 2003). While the overall efficacy is unknown at this time, the immunologic parameters in these studies demonstrate a further disconnect between B cell depletion and reduction of anti-dsDNA antibodies. Interestingly, these patients have a large percentage of circulating plasmablasts ($CD27^{++}CD20^{-}$), memory ($CD27^{+}CD20^{+}$), and/or germinal center ($CD38^{+}CD20^{+}$) B cells. Analysis of the effects of Rituxan on the survival of these B cell subsets will likely provide greater insights into the half-life and survival factors that regulate these germinal center (GC)-derived cells.

B cell differentiation into Ig-secreting plasma cells has been elegantly explored in humans through the investigation of the CD40/CD40L axis (Arpin et al., 1995). GCs represents a major site to permit crosstalk of antigen-activated T, B, and follicular dendritic cells to promote T and B cell expansion and effector functions. In particular, the CD40/CD40L axis is critical within these GC processes for appropriate generation of memory B cells and long-lived $CD38^{++}CD27^{++}$ antibody-forming cells (plasmacytes). Blockade of the CD40/CD40L axis inhibits activation-induced cytidine deaminase-mediated somatic hypermutation, Ig class switch, GC formation, and maintenance and the generation of long-term B cell memory. Patients with inherited mutations of CD40L are unable to undergo Ig class switch and develop elevated levels of IgM but reduced levels of serum IgG and IgA.

Selective patients with active SLE have significantly elevated numbers of circulating $CD38^{+}IgD^{+}$ preswitch GC cells, $CD38^{+}IgD^{-}$ postswitch GC cells, $CD38^{-}CD27^{+}$ memory cells, and $CD38^{++}$ plasma cells. Some of these cells have variable levels of inappropriate CD40L expression and in vitro can undergo spontaneous proliferation and Ig secretion that is blocked with an anti-CD40L mAb (Grammer et al., 2003; Huang et al., 2002). Treatment of SLE patients with an anti-CD40L antibody (BG9588, 5c8, Biogen-IDEC, Inc.) resulted in substantial decreases in anti-ds DNA antibody, proteinuria, and improved clinical SLEDAI score (a conglomerate measure of clinical and laboratory parameters relevant to the severity of SLE disease). Correspondingly, decreases in $CD38^{+}IgD^{+}$ preswitch GC cells and $CD38^{++}$ plasmacytes with less dramatic effects on $CD38^{+}IgD^{-}$ postswitch GC cells were observed in treated patients. Hence, CD40/CD40L blockade may operate at multiple levels, including inhibiting GC initiation, differentiation of GC cells to $CD38^{++}$ plasmacytes, and interfere with GC maintenance in autoantibody generation.

Together, the body of emerging clinical data involving B cell depletion and modulation in a number of human diseases all indicate a clear pathogenic role for B lineage cells, effector functions, and products in disease pathogenesis. Independent of the direct and indirect roles for antibodies in disease, these data also demonstrate that self-reactive B cells and their products likely exhibit distinct properties as compared to non-self-reactive B cells. Additionally, studies of the CD40/CD40L axis further indicate that targeting of specific subsets of downstream B cell effector function is feasible and will be of benefit in certain human autoimmune disorders.

Mechanisms of B Cell Pathogenicity—Humans and Mice

In this final section, we will link observations on B cell effector functions in human disease with those in murine models of disease and discuss how these parallels might aid in understanding the mechanisms of action for B cell depleting therapies in human autoimmunity. We will begin by continuing the theme of potential differences in the precursors of self- and non-self-reactive antibodies, then discuss non-antibody-dependent contribution of B cells as antigen-presenting cells, and finally, review the role of B cells in lymphogenesis and ectopic neolymphogenesis in disease pathogenesis.

Plasma Cells

B cell pathogenesis in autoimmune diseases has been historically attributed to autoantibodies that, in either soluble or IC forms, are thought to initiate local inflammatory cascades. The plasma cell source of these autoantibodies is much less well characterized in humans with respect to exact phenotype, location, or longevity (Arce et al., 2002; Hauser et al., 2003). Populations of long-lived plasma cells have been well characterized in response against foreign antigens (Manz et al., 1997; Traggiai et al., 2003). These cells, in both mice and humans, are enriched in the bone marrow environment. The existence of long-lived autoimmune plasma cells has been demonstrated in murine models of SLE and suggested in humans. Analysis of these cells in humans, however, has proven difficult due to the extreme low frequency and poor understanding of their precursors and intermediates. Although long- and short-lived terminally differentiated plasma cells downregulate surface expression of CD20, and hence would not be depleted by anti-CD20 therapies, very little is known about their immediate precursors in autoimmune diseases. The minimal decreases observed in total serum Ig and more drastic reductions in autoantibody levels following Rituxan treatment suggest either different precursor sensitivities to depletion or, alternatively, that autoantibody-producing plasma cells are enriched in shorter-lived subsets.

Regardless of whether autoantibodies originate from long- or short-lived precursors, it is widely accepted that most pathogenic autoantibodies increase their affinity through somatic mutations. For normal immune responses, this process occurs within the specialized GC environment. Recent data from a murine model of RF autoantibody generation, however, suggests that there are situations in which high-rate somatic mutations can be generated outside of the GC (William et al., 2002). Microdissection of dividing cell foci demonstrate that mutations are readily detected at the T cell zone—red pulp interface in immunoglobulin transgenic MLR/lpr mice, where the normal Fas/FasL mechanisms for maintaining tolerance are compromised. Paralleling these observations, patients with Fas/FasL pathway defects (human autoimmune lymphoproliferative syndrome), with defects in B and T cell apoptosis, develop in both cellular and humoral autoimmunity.

The exact cellular source of autoantibodies in autoimmune diseases becomes important when one tries to explain the large variability seen in autoantibody reduction in patients. Treatment of nonhuman primates with clinically relevant doses of anti-CD20 antibodies demonstrates significantly different rates for depletion of circulating and lymphoid-laden B cells (Reff et al., 1994; Schroder et al., 2003). Additionally, there appears to be differential sensitivities to depletion of various B cell subsets (Vugmeyster et al., 2003). It is conceivable that the variable effects on autoantibodies may result from the differential depletion of these self- and non-self-reactive plasma cell precursors.

Antibody-Independent Effector Functions

The antibody-independent contributions of B cells in human autoimmunity have also been paralleled in mu-

rine models of disease. The ability of B cells to alter T cell activation and expansion was suggested by the generation of an IgH Tg NZB/W SLE-prone mouse strain expressing a germline immunoglobulin (Ig) transgene (Wellmann et al., 2001). Despite a restricted B cell repertoire, these Ig transgenic mice developed high titers of anti-dsDNA autoantibodies but not IC deposits or glomerulonephritis. They also had improved mortality, when compared to their NZB/W littermates, and displayed reduced T cell activation. A similar finding of B cell-mediated disease has also been demonstrated in the New Zealand obese (NZO) diabetes-prone mouse (Haskell et al., 2002). Hence, autoimmune B cells appear capable of driving T cells to mediate certain subsets of autoimmune manifestations. These distinct contributions are consistent with the identification of genetic elements in the NZM2140 SLE-prone mouse model in which clinical and serologic manifestations of disease can be linked, in part, to T (the *sle3* locus), B (*sle2*), and B-T components (*sle1*) (Nguyen et al., 2002).

The contributions of antibody-independent mechanisms to B cell function is further illustrated in MLR/lpr mice made deficient in their ability to secrete Ig but with intact membrane Ig B cells and B cell effector functions—mIgM MRL/lpr (Chan et al., 1999). Despite the absence of secreted Ig and anti-ds DNA antibodies, these mice still develop interstitial nephritis, vasculitis, and less, but still significant, glomerulonephritis and mortality when compared with secretory sufficient MLR/lpr. In contrast, JHD MLR/lpr mice rendered completely B cell-deficient exhibit minimal disease and mortality (Shlomchik et al., 1994). Hence, effector B cell functions, independent of secreted antibody, are also of paramount importance in disease development. A favored hypothesis to explain these antibody-independent effects is the ability of B cells to present antigen to T cells as part of the pathogenic process. Such a possibility clearly exists, though it should be pointed out that these experimental systems utilize immunoglobulin heavy chain transgenes that can limit the autoreactive B cell receptor repertoire.

Direct inhibition of B/T collaboration, through CD40/CD40L blockade, has been demonstrated to be beneficial in several mouse models of lupus. Anti-CD40L treatment blocks both Ig class switching and somatic mutation, reduces anti-dsDNA IgG antibodies, and induces a variable period of general B and T cell unresponsiveness (Kalled et al., 2001; Wang et al., 2003). In addition, CD40 is required for the development of CD8 memory T cell responses (Bourgeois et al., 2002). Together, these data demonstrate multiple mechanisms by which B/T interaction, presumably through antigen presentation or various cosignals and cytokines, may promote T cell effector functions in disease pathogenesis. Additionally, in MLR/lpr mice with B cells lacking β2-microglobulin, and hence MHC class I expression, the contribution of B cells to CD8 T cell activation can also occur independent of antigen crosspresentation (self-antigen presented on MHC class I to CD8 T cells) (Chan and Shlomchik, 2000). Together, these data open the possibility of APC-dependent and APC-independent mechanisms for B cells in autoimmune mouse models of lupus and potentially in human disease.

Lymphogenesis and Neolymphogenesis

The newly recognized role of B cells in lymphogenesis and ectopic neolymphogenesis may also contribute to autoimmunity and serve as a therapeutic mechanism by which B cell depletion or modulation improves human autoimmunity. Dissection of attraction, migration, and retention signals have demonstrated that lymphoid organs and structures are highly dependent on networks of TNF family molecules, integrins, and chemokines (Fu et al., 1997; Cyster, 1999; Ansel and Cyster, 2001). In generating appropriate lymphoid architecture, B cells play an early requisite role through their interactions with organ stroma and provide a milieu permissive for secondary seeding by T cells and dendritic cells. This sequence of events for neolymphogenesis was elegantly demonstrated by the ectopic introduction of a B cell chemoattractant in the pancreas that initiates a cascade of cellular and developmental events leading to the creation of a new lymphoid structure (Luther et al., 2000). A similar dependence on B cells has also been demonstrated in splenic organogenesis as well as normal M cell development in the gut (Ngo et al., 2001; Golovkina et al., 1999). The complementary scenario has been attempted by pancreatic expression of a T cell chemoattractant. However, in this parallel experimental system, lymphoid organogenesis did not progress to the same stage as with B cells, demonstrating a cellular hierarchy in lymphogenesis (Luther et al., 2002).

B cells appear not only important for the formation but also maintenance of these structures. These observations on lymphogenesis formation and maintenance may be important to the recent description of lymphoid-like follicles in joints and inflamed glands in patients with RA and Sjogren's syndrome, respectively (Weyand and Goronzy, 2003; Groom et al., 2002). Hence, B cell depletion or CD40/CD40L blockade could lead to dissolution of these organized pathogenic lymphoid infiltrates and interfere with maintenance of long-lasting T helper memory as well as dendritic cell localization and function (Moulin et al., 2000; Ngo et al., 2001). Looking at the B cell effector mechanisms in total, it is possible that interrupting all or a combination of the above-described pathways is required for clinical efficacy. Further dissection of these mechanisms in ongoing clinical trials as well as complex models will be required to fully appreciate the multiple B cell functions in immunity and disease.

Future of B Cell Therapies

A final point of discussion for human clinical efficacy in autoimmune diseases is the potential lack of complete anti-CD20 B cell depletion in secondary lymphoid organs. Current protocols of Rituxan administration result in an excess of drug that should efficiently deplete all $CD20^+$ B cells. Explanations for the lack of complete depletion, as has been observed in nonhuman primate studies, could be diverse and span from intrinsic B cell resistance as well as factors derived from the local microenvironment (Reff et al., 1994; Schroder et al., 2003). While intrinsic B cell resistance has been more difficult to address and manipulate in humans, the local B cell microenvironment with the multitude of cells and survival signals is of prime importance for additional clinical intervention. It is conceivable that additional costimula-

tory signals, such as CD40/CD40L, with effects on B cell differentiation and survival could rescue them from depletion. Such survival signals within these microenvironments would not only protect B cells from death but could provide additional growth signals for the expansion of autoimmune B cells. In addition to CD40/CD40L, blocking additional survival signals, such as BLys/BAFF and Toll-like receptors (TLR), would likely be beneficial for targeting downstream effector functions, particularly for autoimmune diseases in which these signals are deregulated.

Patients with SLE and Sjogren's syndrome have elevated levels of serum BLys/BAFF (Zhang et al., 2001; Cheema et al., 2001; Groom et al., 2002; Stohl et al., 2003; Mariette et al., 2003). As BLys/BAFF induces B cell proliferation in vitro and in vivo, blockade of this pathway results in loss of follicular and marginal zone B cells (Moore et al., 1999; Thompson et al., 2000). Moreover, mice overexpressing BLys/BAFF develop autoantibodies, Sjogren's syndrome, and SLE-like autoimmunity (Gross et al., 2000; Khare et al., 2000; Yan et al., 2000). In turn, blockade of this survival signal in murine models of SLE results in significant delay in autoantibody development, decreased proteinuria, and improved mortality (Yan et al., 2001; Gross et al., 2001). Analysis of immunologic parameters in SLE and RA patients presently treated with BLys/BAFF blockade will provide additional insights into human B cell functions (Baker et al., 2003). Should BLys/BAFF prove pivotal in human autoimmune B cell survival in vivo, combination therapies with anti-CD40L, anti-CD20, and BLys/BAFF blockade may provide improved efficacy by modulating distinct effector mechanisms.

TLR ligands, particularly for TLR9 (CpG oligonucleotides, hypomethylated bacterial, and mammalian DNA), are potent activators of dendritic cells but function as cosignals for long-term serologic memory B cells and self-reactive transgenic B cells (Bernasconi et al., 2002; Leadbetter et al., 2002; Viglianti et al., 2003). In addition, activation of TLR9 in vivo accelerates nephritis in the MLR/lpr lupus mouse model and exacerbates experimental allergic encephalitis (Ichikawa et al., 2002; Anders et al., 2004). Consistent with the notion that bacterial or viral infections may act as triggers for clinical flares of autoimmune disorders, triggering of TLR4 (LPS) in mice prone to the development of myocarditis induces clinical autoimmune myocarditis (Eriksson et al., 2003). Hence, activation of TLRs in dendritic cells and B cells may contribute in disease pathogenesis. Interestingly, ongoing clinical studies are utlizing CPG and CPG-conjugated allergens to rebalance a deregulated autoimmune system in the treatment of asthma and other autoimmune disorders (Krieg, 2003). Whether TLRs are differentially utilized among immune and autoimmune B cells is unclear at this time. These ongoing clinical studies combined with preclinical insights in TLR biology should inform us whether this axis can be harnessed in the treatment of autoimmunity.

The beginning of this first decade of the 21st century has been an exciting time with the emergence of a multitude of therapeutic probes to understand human B cell biology. These studies have not only reinforced the importance of autoantibodies and ICs in disease pathogenesis but also provide additional insights into the biology of self-reactive B lineage cells. The second half of this first decade promises to be even more exciting as the impact of these emerging B cell-directed therapies in disease and their immunologic consequences will likely not only improve patient survival and quality of life but will undoubtedly illuminate the multitude of B cell functions in human immunity and autoimmunity.

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Review

Strategies of therapeutic complement inhibition

Tom E. Mollnes [a,*], Michael Kirschfink [b]

[a] *Institute of Immunology, Rikshospitalet University Hospital and University of Oslo, N-0027 Oslo, Norway*
[b] *Institute of Immunology, University of Heidelberg, Germany*

Abstract

The involvement of complement in the pathogenesis of a great number of partly life threatening diseases defines the importance to develop inhibitors which specifically interfere with its deleterious action. Endogenous soluble complement-inhibitors, antibodies or low molecular weight antagonists, either blocking key proteins of the cascade reaction or neutralizing the action of the complement-derived anaphylatoxins have successfully been tested in various animal models over the past years. Promising results consequently led to first clinical trials. This review is focused on different approaches for the development of inhibitors, on their site of action in the cascade, on possible indications for complement inhibition based on experimental animal data, and on potential side effects of such treatment.
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Keywords: Complement; Inflammatory disorders; Inhibitors; Therapy

1. Introduction

Complement as an essential component of the immune system is of substantial relevance for the destruction of invading microorganisms and for maintaining tissue homeostasis including the protection against autoimmune diseases (Walport, 2001a,b). However, excessive or uncontrolled complement activation significantly contributes to undesired tissue damage. Following complement activation, biologically active peptides, such as C5a and C3a elicit a number of proinflammatory effects, including the recruitment of leukocytes, degranulation of phagocytic cells, mast cells and basophils, smooth muscle contraction, and increase of vascular permeability. Upon complement-dependent cell activation, the inflammatory response is further amplified by subsequent generation of toxic oxygen radicals and the induction of synthesis and release of arachidonic acid metabolites and cytokines. Consequently, complement activation presents a considerable risk of harming the host by directly and indirectly mediating inflammatory tissue destruction.

Under physiological conditions, activation of complement is effectively controlled by the coordinated action of soluble as well as membrane-associated regulatory proteins. Soluble complement regulators, such as C1-inhibitor, anaphylotoxin inhibitor (serum carboxypeptidase N), C4b binding protein (C4BP), factors H and I, clusterin and S-protein (vitronectin), restrict the action of complement in body fluids at multiple sites of the cascade reaction. In addition, each individual cell is protected against the attack of homologous complement by surface proteins, such as the complement receptor 1 (CR1, CD35), the membrane cofactor protein (MCP, CD46) as well as by the glycosylphosphatidylinositol (GPI)-anchored proteins, decay-accelerating factor (DAF, CD55) and CD59. When complement is improperly activated, these regulatory mechanisms may be overwhelmed, resulting in tissue destruction and disease.

Clinical and experimental evidence underlines the prominent role of complement in the pathogenesis of numerous inflammatory diseases (Szebeni, 2004). The list of such conditions is rapidly growing, including immune-complex diseases such as rheumatoid arthritis and systemic lupus erythematosus, ischemia–reperfusion (I/R) injury locally manifested as infarctions or systemically as a post-ischemic inflammatory syndrome, systemic inflammatory response

* Corresponding author. Tel.: +47 90630015; fax: +47 23073510.
E-mail address: t.e.mollnes@labmed.uio.no (T.E. Mollnes).

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syndrome (SIRS) and acute respiratory distress syndrome (ARDS), septic shock, trauma, burns, acid aspiration to the lungs, renal diseases, inflammatory and degenerative diseases in the nervous system, arteriosclerosis, transplant rejection and inflammatory complications seen after cardiopulmonary bypass and haemodialysis. In principle, when inflammation is involved in the pathogenesis, complement has to be considered as a possible mediator in the disease process (Mollnes et al., 2002).

In recent years, great progress has been made in complement analysis to better define disease severity, evolution and response to therapy. Modern diagnostic technologies which focus on the quantification of complement activation products now provide a comprehensive insight into the activation state of the system. Thus, to focus on complement inhibition appears to be a logical approach in order to arrest the process of inflammatory disorders. Due to the vast amount of data already available in the literature, only parts of it can be included here. For further reading, see previous general reviews, e.g. (Lambris and Holers, 2000; Asghar and Pasch, 2000; Kirschfink, 2001; Quigg, 2002; Morgan and Harris, 2003; Mollnes, 2004). In addition, a number of review papers dealing either with a certain candidate inhibitor or treatment of a certain disease have been published the last decade.

2. General aspects of complement inhibition therapy

2.1. Complement in inflammation

The pathophysiology of inflammation is complex and diverse with innumerable mutually interacting mediators (see also preceding reviews in this issue). The role of complement in the inflammatory network may also vary with the different conditions, from being crucial to just epiphenomenonal. An essential issue is whether complement activation, by activating leukocytes, endothelial cells, platelets and other cells, induces secondary inflammatory mediators which may contribute to or even determine tissue damage, such as cytokines, reactive oxygen species, arachidonic acid metabolites and expression of adhesion molecules. On the other hand, some of these mediators may be primarily induced and activate complement as a secondary mechanism of inflammation. The question frequently raised is what comes first, the chicken or the egg. This has significant implication to the rationale and design of anti-inflammatory therapy in general and for complement inhibition in particular. In principle, it would be most effective to block upstream of the inflammation cascade, e.g. the primary inducer(s). The matter is, however, rather complex since some of the inflammatory mediators may have mainly adverse effects on tissue homeostasis whereas others are beneficial. Thus, a careful dissection of the pro and anti-inflammatory effects of complement is needed to identify and inhibit those mediators contributing to the tissue damage and to spare those which are needed for a balanced homeostasis.

2.2. Rationale for complement inhibition in human disease

Targeting complement as a therapeutic goal requires detailed knowledge about the activation mechanisms and mediators responsible for the inflammation, enabling optimal inhibitory treatment for the actual condition. Many mechanisms have been experimentally elucidated, particularly in I/R injury, but the field is still in its infancy. Successful treatment with a specific complement-inhibitor is the ultimate proof that complement plays an essential role in the actual disease. The design of clinical trials has to rely on data from animal disease models. However, due to species differences, results from animal studies are not necessarily applicable to humans. Finally, only their clinical application determines to what extent a particular disease will benefit from such treatment. Only comparable to primary deficiency states as experiments of nature, specific therapeutic inhibition will provide the ultimate concept validation for the role of complement in the pathogenesis of human diseases.

Activation of complement, as detected by increased levels of complement activation products in plasma samples, is known to occur in a number of disease conditions. However, increased complement activation does not necessarily imply that complement is of importance in the pathogenesis. On the other hand, normal systemic levels of activation products is seen in many conditions where local complement activation is likely to play a role in local tissue damage. In such conditions, complement activation can be detected in tissue biopsies or in the local body fluid. In general, an ongoing systemic activation is required for increased activation products to be detected in a plasma sample. The site of activation will determine the inhibitor to be used and its route of application.

2.3. Potential sites in the complement cascade to be inhibited (Fig. 1)

The majority of approaches to inhibit complement has focused on either blocking at the level of C3 implying a general and broad inhibition of the system, or on selective blocking of C5 activation with subsequent inhibition of C5a and C5b-9 (TCC) formation (Fig. 1). In favor of the former is the argument that if complement activation is detrimental, it should be reasonable to extend the system's inhibition as wide and complete as possible. On the other hand, blocking of the terminal pathway allows C3 activation as required to mount an effective defense against foreign invaders. In fact, both arguments over-simplify the situation and have limited clinical validation. The mechanisms of complement activation and – even more – the contribution of this activation to the pathophysiology of different clinical conditions are so diverse that a differential approximation to this issue is required and several strategies must be considered.

One such strategy will be to target the initiation step of complement activation, provided detailed knowledge on the activation mechanism. Each of the three initial activation

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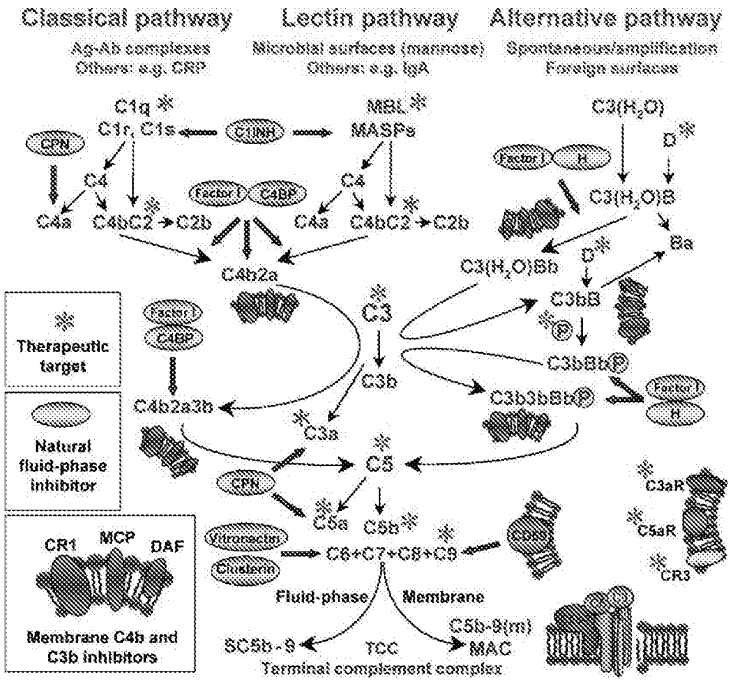

Fig. 1. Complement cascade reaction and physiological regulation. Various reagents with potential therapeutic applications have been developed to target complement activation and function (potential targets are indicated by red asterisks). For details, see Mollnes et al. (2002), where the figure was originally published (reprinted here with permission from Elsevier).

pathways can be blocked separately. For example, inhibition of factor D, the rate limiting step of the alternative pathway, is an attractive approach in inhibiting alternative pathway activation. It should be noted that alternative pathway activation may either directly be triggered or induced by classical or lectin pathway activation for amplification. Whereas inhibition of alternative pathway could block the amplification of complement activation, certain important classical and lectin pathway functions, e.g. C1q or MBL mediated opsonisation and receptor binding, remains intact. In certain conditions of systemic complement activation, such as in ischemia reperfusion injury, trauma or sepsis, the alternative pathway amplification loop may be responsible for an uncontrolled and detrimental activation, irrespective of the initial trigger.

Another strategy relies on targeting specific activation products or their respective receptors, particularly the anaphylotoxins C3a or C5a. In airway hyper-responsiveness, there may be an indication for selective blocking of C3a function, which can be achieved by anti-C3a antibodies or C3aR antagonists, thereby preserving other important C3 functions. Similarly, as C5a is considered the main contributor to the pathophysiology of inflammations associated with systemic complement activation, its function should be selectively blocked, leaving the C5b-9 pathway open for killing of bacteria such as Neisseria.

The main challenge for developing effective and safe anti-complement therapeutics is to balance the beneficial effects obtained by the inhibition with the preservation of sufficient functional activity for anti-microbial protection and for tissue homeostasis.

2.4. Possible adverse effects of complement inhibition

Adverse effects resulting from complement inhibition may be directly related to the function of complement, i.e. increased susceptibility to infection and autoimmune- and immune-complex diseases, arising from impaired opsonisation, adaptive immune response, tolerance or elimination of immune-complexes. To date, such complications have not been observed in animal models. This is best explained by either incomplete inhibition and/or restriction to short-term interventions. In fact, 60% inhibition of complement activity appears to bed sufficient for treatment of collagen-induced arthritis (Wang et al., 1995). This is most important when considering long-term treatment in chronic diseases. A certain degree of inhibition may be sufficient to reduce detrimental effects of complement activation, though defense mechanisms may still be preserved. The risk of infectious complications is most probably highest when blocking C3. The redundancy of the three activation pathways may reduce the risk of infection if only one pathway is selectively blocked. Blocking of C5b-9 formation, however, could lead to increased susceptibility to certain pathogens, such as Neisseriae. Based on experimental studies, Gram-negative septic shock is one of the conditions that may benefit from com-

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plement inhibition. In these cases, treatment with antibiotics would compensate for short-term complement inhibition.

A novel role for complement in tissue regeneration has recently been demonstrated (Mastellos and Lambris, 2002). It is unknown whether impairment of this function will be a consequence of long-term complement inhibition.

Inhibitors which are immunogenic may rapidly loose their efficacy. The risk of immune response is significantly reduced by the use of recombinant human proteins, small molecular inhibitors and humanized antibodies, as discussed below.

2.5. Mode of administration, clearance and long-time treatment

The administration route is critical for patients suffering from chronic diseases requiring long-time therapy. Here, oral, nasal or rectal application is definitely superior to intravenous injection, whereas acute, severe disorders are preferentially treated through the intravenous route. Furthermore, although systemic diseases like sepsis and SIRS may require systemic treatment, diseases with organ specific damage, such as glomerulonephritis, central nervous system diseases and rheumatoid arthritis may benefit from local application. Recombinant proteins and small molecular inhibitors, in contrast to antibodies, generally have a short half-life and are best suited for treatment of acute conditions. Their modifications, e.g. by conjugation to human immunoglobulin Fc fragments, have successfully increased half-life (Harris et al., 2002).

2.6. Targeted application

Targeted application aims at to limit potential systemic side effects. This approach is indicated if the inflammatory process is clearly organ specific and systemic fluid-phase activation can be excluded.

Targeting may be nonspecific, directed to any membrane, or specific, directed to certain cells or organs. One approach for nonspecific targeting is the membrane "tagging", obtained by coupling the inhibitor to a lipid tail. This tail will bind to membranes undergoing internal–external changes, as illustrated by the myristoyl-electrostatic switch paradigm where a truncated form of sCR1 (APT070) was used (Smith and Smith, 2001; Smith, 2002). This agent proved to be 100-fold more potent than the parent molecule and has been used for treatment of experimental rheumatoid arthritis (Linton et al., 2000). Based on the promising experimental results, the agent has been tested in volunteers. Clinical studies in patients with rheumatoid arthritis are underway (Smith, 2002). sCD59 has also been "tagged" in a similar manner, being 100-fold more potent than sCD59 (Smith and Smith, 2001). The rodent complement C3-inhibitor Crry (complement receptor 1 related gene/protein) has been "tagged" using the same approach in experimental animal studies (Fraser et al., 2002).

Another approach for targeted application relies on the antigen-specificity of antibody-conjugates. DAF was successfully targeted to the surface of Chinese hamster ovary cells by a conjugate of DAF and IgG anti-danasyl antibody (Zhang et al., 2001). A similar antigen-targeted form of sCD59 has been produced (Zhang et al., 1999). Recently, conjugates were made between a CR2 fragment, recognizing sites of C3 activation, and DAF and CD59 (Song et al., 2003). These conjugates bound to C3 opsonised cells and were 20-fold more efficient than the untargeted molecules in inhibiting complement activation. In a mouse model of lupus nephritis, the authors showed that CR2-DAF but not soluble DAF bound to the kidney. Direct proximal tubule targeted complement inhibition using single chain antibodies conjugated to complement-inhibitors efficiently attenuated experimental nephrotic syndrome and established a key role for C5b-9 in the pathogenesis (He et al., 2005).

A third approach for targeted inhibition is conjugating the complement-inhibitor to another inflammation-modulating molecule, exemplified by the sCR1-sLe(x) (TP20) molecule (Picard et al., 2000). sLe(x) is a ligand for E- and P-selectin and thus TP20 combines inhibition of complement and leukocyte adhesion (Rittershaus et al., 1999). TP20 was found to be superior to unconjugated sCR1 (TP-10) in reducing tissue damage in an experimental model of cerebral stroke (Huang et al., 1999), in immune-complex mediated lung injury (Mulligan et al., 1999), and in I/R injury in experimental allogenic lung transplantation (Stammberger et al., 2000). Furthermore, TP20 reduced myocardial (Zacharowski et al., 1999) and skeletal muscle I/R injury (Kyriakides et al., 2001a) and moderated acid aspiration injury in mice (Kyriakides et al., 2001b).

2.7. Prodrugs

A prodrug is inactive or has low activity until it reaches a site where it is activated. Although fusion of DAF or CD59 with human immunoglobulin Fc domains markedly extended the half-life (Quigg et al., 1998b; Rehrig et al., 2001; Harris et al., 2002) of the construct, it was found that the biological activity of both regulators was reduced. Full activity was retained upon enzymatic release of the Ig moiety. This principle was utilized to develop a novel DAF fusion protein with virtually no biologic activity. The construct contains a site which is sensible for enzymatic cleavage by a metalloproteinase. After enzymatic cleavage, restricted to sites of inflammation, the biologic activity of DAF was restored (Harris et al., 2003).

2.8. Gene therapy

A transgene strategy for delivery of rat complement regulators using adenovirus vectors has been developed (McGrath et al., 1999). Local production of the complement-inhibitor Crry by astrocytes was found to attenuate experimental allergic encephaloymyelitis (Davoust et al., 1999). sCR1 was delivered by retrovirally transfected cells or by naked DNA directly to the joint and prevented progression of collagen-induced arthritis (Dreja et al., 2000). A similar protective

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effect on antibody-mediated glomerular injury was achieved upon the systemic release of Crry in transgenic mice (Quigg et al., 1998a).

Hyperacute xenotransplant rejection is caused by binding of naturally occurring antibodies and subsequent complement activation. In order to overcome this complement-mediated rejection various transgenic animals expressing human membrane complement regulators have been developed for use in xenotransplantation (Mccurry et al., 1995; Cozzi and White, 1995).

This issue has been extensively described elsewhere.

3. Specific aspects: inhibitors and their application

The complement system is normally kept under strict control by fluid-phase and membrane-bound regulatory proteins. The need for keeping this system under control is illustrated by the fact that there are as many regulators as there are ordinary components, and that deficiency of a regulatory protein is associated with substantially disturbed homeostasis. All complement regulators are inhibitors of activation, except for properdin, which stabilises the alternative C3 convertase (C3bBbP) and thus enhance activation. Many of the candidate drugs for therapeutic complement inhibition are either naturally occurring complement-inhibitory proteins or derivatives thereof.

3.1. Naturally occurring regulators

3.1.1. C1-inhibitor

C1-inhibitor is a naturally occurring serine protease inhibitor and the only known inhibitor of C1r and C1s, and controls MASP-1 and MASP-2 of the lectin pathway as well (Matsushita et al., 2000). Recently, a novel inhibitory function on the alternative pathway was documented (Jiang et al., 2001). Thus, an effect of C1-inhibitor on complement may principally be mediated through either of the initial pathways. Furthermore, the action of C1-inhibitor is not restricted to complement but has a broad spectrum of targets including factor XIIa, kallikrein and factor XIa. C1-inhibitor is available for clinical use as substitution therapy in hereditary angioedema (HAE). Notably, the pathophysiology of HAE is closely related to the release of bradykinin and the main effect of C1-inhibitor is to reduce bradykinin formation through inhibition of the kallikrein/kinin system. From this point of view, HAE is not a complement-mediated disease, and it should be emphasized that the effect of C1-inhibitor, when used for therapeutic treatment, is not necessarily complement-dependent, but may well be explained by inhibition of other proteins. C1-inhibitor has been widely used in a number of clinical conditions. The principle of supra-physiological doses to obtain more efficient regulation has been applied, although in some cases the treatment may be regarded as substitution for acquired low concentrations. Clinical conditions for C1-inhibitor treatment include sepsis, burns, capillary leak syndrome associated with bone marrow transplantation and IL-2 therapy, myocardial infarction, trauma and transplantation (for review, see Kirschfink and Mollnes, 2001). Unfortunately, controlled studies are still missing and except for C1-inhibitor deficiency there is no accepted indication for this treatment.

3.2. Recombinant complement regulatory proteins

The family of regulators of complement activation (RCA) comprises CR1 (CD35), CR2 (CD21), MCP (CD46), DAF (CD55), factor H and C4BP. They are all powerful inhibitors of C3 and C5 convertases, except for CR2, which may play a minor role in regulation of complement. If an extensive inhibition of complement is required, the RCA proteins are candidates since they all interfere with activity of the C3 and C5 convertases.

3.2.1. C4BP and factor H

C4BP and factor H are soluble regulators of the classical and alternative C3/C5 convertases, respectively. Recombinant factor H has been produced (Sharma and Pangburn, 1994). Using a glycosyl-phosphatidyl inositol (GPI) anchor, a membrane form of C4BP was constructed which protected porcine endothelial cells against attack by human complement (Mikata et al., 1998a). A similar approach was used to bind factors H and I to a xenosurface (Yoshitatsu et al., 1999). Binding of factor H to an artificial surface abrogated the surface-induced complement activation and thereby improved biocompatibility (Andersson et al., 2001). C4BP and factor H have not been used clinically.

3.2.2. Crry (complement receptor 1 related gene/protein)

The rodent C3 convertase inhibitor Crry, which has both decay-accelerating and cofactor activity, resembles CR1 and has been used in two different strategies in mouse models. First, a soluble Crry was constructed and coupled to an immunoglobulin tail for injection (Crry-Ig) (Quigg et al., 1998b). Second, Crry was over-expressed as a non-immunogenic soluble protein in the animal under the control of a widely expressed transgene (Quigg et al., 1998a). Both approaches protected mice against experimentally induce acute glomerulonephritis. Furthermore, transgenic expression of Crry in mice developing SLE (MRL/lpr mice) prolonged survival and attenuated renal damage (Bao et al., 2002), later confirmed by treatment with the soluble Crry-Ig protein (Bao et al., 2003). Soluble Crry was also shown to inhibit intestinal I/R injury in mice even when administered 30 min after start of reperfusion (Rehrig et al., 2001). It has recently become evident that control of complement activation is also of crucial importance for the placenta barrier homeostasis where complement activation may induce fetal loss (see Girardi et al., this issue). Thus, in a mouse model of anti-phospholipid antibody-induced fetal loss, soluble Crry was protective (Holers et al., 2002).

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3.2.3. Soluble CR1

CR1 is the only member of the RCA family with cofactor activity and decay-accelerating activity in both the classical and alternative pathway (C4b and C3b). A soluble form of CR1 (sCR1) was constructed by deleting the transmembrane part of the molecule (Weisman et al., 1990). sCR1 inhibited both classical and alternative pathway activation in concentrations equivalent to 1% of physiological C4BP and factor H concentration. The protein was demonstrated to reduce experimental myocardial I/R injury by approximately 50%, a cardioprotective effect which has later been confirmed in several studies (Shandelya et al., 1993; Smith et al., 1993; Homeister et al., 1993; Lazar et al., 1999).

The half-life of the original sCR1 was only a few hours. A slightly extended half-life was obtained by fusing sCR1 with an albumin-binding receptor (Makrides et al., 1996). An sCR1-F(ab')2 chimeric protein combined an extended half-life with improved targeting (Kalli et al., 1991). Its parenteral administration is required.

A variant of sCR1, named sCR(desLHR-A), which specifically inhibits the alternative pathway has been constructed by deleting the C4b binding domain (Scesney et al., 1996). This protein attenuated tissue damage in experimental myocardial infarction (Murohara et al., 1995; Gralinski et al., 1996) and reduced the endothelium-dependent relaxation in rabbit tissue, shown to be mediated by C5b-9 (Lennon et al., 1996).

sCR1 has been widely used in a number of animal disease models with convincing effects on tissue damage. In addition to the beneficial effect on myocardial I/R injury described above, the following I/R injuries have been attenuated by sCR1: local and remote injury in rat intestine (Hill et al., 1992; Eror et al., 1999), gut ischemia and endothelial cell function after hemorrhage and resuscitation in rats (Fruchterman et al., 1998), liver injury (Lehmann et al., 1998; Chavez Cartaya et al., 1995), and local and remote (lung) injury in skeletal muscle (Pemberton et al., 1993; Lindsay et al., 1992).

The beneficial effect of sCR1 in autoimmune diseases has been demonstrated in the passive reverse Arthus reaction, a dermal immune-complex mediated vasculitis (Yeh et al., 1991), in experimental arthritis in rats (Goodfellow et al., 1997, 2000), in immune-complex and complement-mediated lung injuries and thermal trauma in rats (Mulligan et al., 1992). Furthermore, allergic reactions (Lima et al., 1997) and pseudoallergic reaction to infusion of liposomes (Szebeni et al., 1999) were attenuated by sCR1. Improvement was also obtained in an experimental rodent model of ARDS (Rabinovici et al., 1992) as well as in a guinea-pig model of asthma (Regal et al., 1993). In mice, the inflammatory reaction induced by acid aspiration to the lungs was markedly improved by sCR1 (Weiser et al., 1997).

sCR1 has also proved to be efficient in treatment of experimental glomerulonephritis (Couser et al., 1995), myasthenia gravis (Piddlesden et al., 1996), as well as in experimental allergic encephalomyelitis (a model for multiple sclerosis) (Piddlesden et al., 1994), autoimmune neuritis in rats (Jung et al., 1995) and traumatic brain injury (Kaczorowski et al., 1995).

The role of complement in xenotransplantation has been indisputable, and therefore, the effects of sCR1 in reducing hyperacute rejection was not surprising (Pruitt et al., 1991, 1994; Xia et al., 1992; Homeister et al., 1993). Incorporating a mini-CR1 coupled to a GPI anchor into porcine cells was protective against human complement (Mikata et al., 1998b). The role of complement in allotransplant rejection is less clear, but the beneficial effects observed upon sCR1 treatment in various allotransplant models has highlighted complement as an important mechanism in allotransplant rejection as well (Pruitt and Bollinger, 1991; Pratt et al., 1996; Lehmann et al., 1998; Pierre et al., 1998).

Extracorporeal circulation like haemodialysis and cardiopulmonary bypass (CPB) is associated with a complement-dependent systemic inflammatory response. sCR1 reduced lung injury and pulmonary hypertension in pigs undergoing CPB (Gillinov et al., 1993) and inhibited complement and leukocyte activation in a simulated extracorporeal circulation setup (Larsson et al., 1997).

sCR1 has been developed into a pharmaceutical preparation (TP-10; Avant Immunotherapeutics Inc., Needham, MA). It has been tested in patients with ARDS, acute myocardial infarction, lung transplantation and post-cardipulmonary bypass syndrome (Rioux, 2001). The results of the ARDS study showed an efficient inhibition of complement activation, but there was no significant difference between the clinical outcome in the groups (Zimmerman et al., 2000). A pharmacokinetic study of TP-10 in infants undergoing cardiopulmonary bypass showed that administration of the drug was safe and indicated a protective effect on vascular function (Li et al., 2004). In a multi-centre study of patients undergoing cardiopulmonary bypass there was no difference between the test and the placebo group with respect to primary end-point, but a significant reduction in mortality and myocardial infarction was observed in males (Lazar et al., 2004). Interestingly, treatment of lung transplant patients with TP-10 significantly reduced the time on mechanical ventilation compared with placebo (Keshavjee et al., 2005).

3.2.4. Soluble DAF and MCP

A recombinant soluble form of DAF (sDAF) was constructed and found to inhibit complement both in vitro and in vivo. It attenuated the reverse passive Arthus reaction (Moran et al., 1992), but the lack of factor I cofactor activity may limit its potential for clinical application. Similarly, a recombinant soluble form of MCP (sMCP) has been made (Christiansen et al., 1996b). sMCP attenuated the reverse passive Arthus reaction (Christiansen et al., 1996a) and prolonged hyperacute xenograft rejection (Christiansen et al., 1996b). A fusion protein of sMCP and a soluble form of the low affinity human IgG receptor Fc gamma RII (CD32) was more effective in protection against xenotransplant rejection than the sMCP alone (Lanteri et al., 2000). Comparison between sDAF, sMCP and sCR1 showed that sCR1 was more effective than each of the

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other two in the classical pathway. In the alternative pathway, sCR1 and sMCP had almost the same effect (Christiansen et al., 1996a). The combination of sDAF and sMCP was more effective than each protein alone. A fusion protein of sDAF and sMCP was constructed (CAB-2) which retained both decay acceleration (DAF) and co-factor activity (MCP). CAB-2 was protective in the passive reverse Arthus reaction and in Forssman shock (Higgins et al., 1997), and prolonged pig-to-primate heart xenotransplant survival (Salerno et al., 2002) as well as ex vivo porcine-to-human heart transplant graft survival (Kroshus et al., 2000).

3.2.5. Soluble CD59

A recombinant soluble form of CD59, sCD59, has been constructed, but is rather inefficient in the fluid-phase (Sugita et al., 1994). A fusion protein of sCD59 and sDAF has been constructed (Fodor et al., 1995), with the aim of combining a C3/C5 convertase inhibitor activity with a C5b-9 inhibitor. Its possible therapeutic effect remains to be shown. A soluble form of CD59 has been fused with a soluble form of the rodent C3 convertase inhibitor, Crry, enabling inhibition of rodent complement activation both at the C3 and C9 level (Quigg et al., 2000). A membrane-targeted form of sCD59 with biological activity in vitro and in vivo has also been constructed (Fraser et al., 2003). Selective inhibition of C5b-9 may be indicated in certain forms of glomerulonephritides, arthritides and encephalomyelitides which appears depend mainly on the formation of the membrane attack complex.

3.2.6. Microbial proteins

Microbes frequently express proteins with high homology to human complement regulatory proteins and thus protect themselves against complement attack. One such protein, vaccinia virus complement control protein, has CR1-like activity and has been studied in detail (Jha and Kotwal, 2003). It has been suggested as a candidate molecule for complement therapy. In addition to its complement-inhibitory function, this protein also directly interferes with binding of xenoantibodies to their targets.

3.3. Antibodies

Monoclonal antibodies combine the advantage of being highly specific with a relatively long half-life and allow large-scale production. A main limitation is the need for parenteral administration and the risk of immunization. The latter can be reduced by humanizing the antibody, or by producing human monoclonal antibodies, as recently described for an anti-C5 antibody isolated from a human phage display library (Marzari et al., 2002).

3.3.1. Anti-MBL, -factor D and -factor B

Several recent data indicate that the lectin pathway is of importance in the pathogenesis of I/R injury (Jordan et al., 2001; Fiane et al., 2003; Hart et al., 2005). In experimental septicaemia, MBL-A deficiency improved survival (Takahashi et al., 2002). Anti-MBL antibodies blocking the lectin pathway have been developed (Zhao et al., 2002; Collard et al., 2000). As already outlined above, factor D is the rate limiting step in the alternative pathway which is of pivotal importance for the I/R injury in mice (Stahl et al., 2003), either by direct activation or through the amplification loop. An anti-human factor D monoclonal antibody (mAb 166-32) has been developed (Tanhehco et al., 1999; Fung et al., 2001) and found to inhibit complement and leukocyte activation in baboons undergoing CPB (Undar et al., 2002). Blocking of factor B prevented phospholipid-induced pregnancy loss in mice (Thurman et al., 2005).

3.3.2. Anti-C5

Since there are no natural inhibitors for activated C5, there is a particular need to develop specific C5-inhibitors. Furthermore, since C5 may be activated directly, independent of C3, specific inhibitors are required. Monoclonal antibodies to mouse C5, like the mAb BB5.1 (Frei et al., 1987) were important tools to demonstrate the role of the terminal complement pathway in experimental diseases like collagen-induced arthritis (Wang et al., 1995) and lupus-like nephritis (Wang et al., 1996). Notably, such treatment of collagen-induced arthritis with anti-C5 antibody not only prevented disease onset, but also ameliorated established disease. An anti-rat C5 mAb was found to be protective in experimental myocardial infarction (Vakeva et al., 1998). The anti-human C5 antibody N19/8 (Wurzner et al., 1991) was a breakthrough in this field. It was used to demonstrate inhibition of terminal pathway activation in an artificial cardiopulmonary bypass model where leukocyte and platelet activation was also attenuated (Rinder et al., 1995). It also prevented hyperacute graft rejection in a model of porcine-to-human heart transplantation (Kroshus et al., 1995). Later, several anti-C5 antibodies have been generated. One of these, a humanised single chain fragment formerly designated as h5G1.1-scFv (Thomas et al., 1996) has reached clinical trials as pexelizumab (Alexion Pharmaceuticals, Cheshire, CT) with focus on targeting complement in cardiovascular disease. In a study of patients undergoing CPB, complement inhibition was achieved with subsequent reduced leukocyte activation and postoperative complications, including cognitive defects, myocardial damage and blood loss (Fitch et al., 1999). Although no difference in death or myocardial infarction was found between treated and placebo groups, a beneficial effect on their clinical outcomes was observed in a subgroup of patients with isolated coronary artery bypass grafting (Shernan et al., 2004). In a large multi-centre study of patients on cardiopulmonary bypass no difference was found for the same end-points in patients undergoing coronary artery bypass grafting only, but for the whole patient population a significant reduction in death and myocardial infarction 30 days postoperatively was observed for the pexelizumab group (Verrier et al., 2004). A possible beneficial effect of this drug on neurocognitive function after cardiopulmonary bypass was recently reported (Mathew et al., 2004).

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When this compound was used in patients with myocardial infarction, no reduction in infarct size was observed despite an efficient complement-inhibitory effect (Mahaffey et al., 2003). However, a subgroup of myocardial infarction patients with ST-elevation undergoing percutaneous coronary intervention showed a significantly reduced mortality (Granger et al., 2003). Interestingly, CRP and IL-6, both mediators correlating to mortality, were significantly reduced in this patient group upon treatment with pexelizumab (Theroux et al., 2005).

Eculizumab (Alexion Pharmaceuticals, Cheshire, CT), a fully humanized moab C5-inhibitor has been developed for chronic inflammatory (autoimmune) indications, such as rheumatoid arthritis, membraneous glomerulonephritis, dermatomyositis and lupus. Results from ongoing clinical studies are awaited.

This agent has also been tested in patients with paroxysmal nocturnal hemoglubinuria (PNH), a condition with increased complement sensitivity of red cells due to low expression of DAF and CD59. Less haemolysis and requirements for transfusions were observed (Hillmen et al., 2004).

3.3.3. Anti-C5a

C5a is considered the biologically most potent fragment formed during complement activation, and is therefore, regarded as an important target for inhibition. In animal studies, anti-C5a antibodies reduced myocardial I/R injury (Amsterdam et al., 1995), reduced neutrophil-mediated impairment of endothelium-dependent relaxation after CPB (Tofukuji et al., 1998), reduced local and remote injury in intestinal I/R injury (Wada et al., 2001), improved survival (Czermak et al., 1999; Huber-Lang et al., 2001) and reduced IL-6 production (Hopken et al., 1996) and oxygen demand (Mohr et al., 1998) in sepsis. Blocking C5a also inhibited sepsis-related thymic cell apoptosis (Guo et al., 2000; Riedemann et al., 2002), alleviated symptoms of ARDS in septic primates (Stevens et al., 1986) and reduced lung vascular injury following thermal trauma (Schmid et al., 1997). Recently, anti-C5a antibodies were shown to ameliorate impairment of the coagulation and fibrinolytic system in a rat model of sepsis, emphasizing a possible role of C5a in activation of other plasma cascade systems (Laudes et al., 2002).

A novel approach for C5a inhibition was recently demonstrated using a mAb reacting with the C5a moiety of the C5 molecule without inhibiting C5 cleavage (Fung et al., 2003). Thus, C5a is "pre-neutralised" before it is formed and thereby increases efficacy of C5a neutralization. This antibody has been investigated in a human whole blood model of Neisseria infection, with virtually complete inhibition of the inflammatory reaction while bacterial killing was not affected (Sprong et al., 2003).

3.3.4. Anti-C5-9

Anti-C8, which blocks C5b-9 (TCC) formation without interfering with C5 cleavage, was found to reduce tissue damage in rat hearts perfused with human serum (Rollins et al., 1995). Furthermore, anti-C8 inhibited platelet activation during simulated CPB (Rinder et al., 1999).

3.4. Small molecule inhibitors

3.4.1. C1 binding peptides

Peptides interacting with the function of C1q have been produced from phage display libraries (Lauvrak et al., 1997; Roos et al., 2001). Synthetic peptides interfering with IgG binding to C1 prolonged xenograft survival (Fryer et al., 2000). It should be noted that C1q is not only activated by antibodies, but by several other substances which may be of pathogenic importance in human diseases, like beta-amyloid in Alzheimers disease, and C-reactive protein with possible implications for the pathogensis of atherosclerosis. Inhibition of C1q has recently been extensively reviewed (Roos et al., 2002). A novel highly selective small molecule C1s-inhibitor (C1s-INH-248, Knoll, BASF, Germany) was found to protect against experimental myocardial I/R injury (Buerke et al., 2001).

3.4.2. Compstatin

A 13-residue cyclic peptide was isolated using combinatorial peptide libraries to identify C3 binding peptides (Sahu et al., 1996), later characterised in detail and named compstatin (Morikis et al., 1998). This peptide blocks cleavage of C3 and is highly specific for binding to C3. No interaction with other cascade proteins has been demonstrated. It was found to reduce complement and granulocyte activation in an in vitro model of extracorporeal circulation (Nilsson et al., 1998) and to protect against hyperacute xenograft rejection ex vivo (Fiane et al., 1999). Compstatin works only in primates, and therefore, animal studies are limited. One study showed inhibition of heparin–protamine-induced complement activation in a primate in vivo model (Soulika et al., 2000).

3.4.3. C3aR antagonists

A non-peptide C3aR antagonist (SB 290157) blocking human C3aR also antagonises rodent C3aR and was found to reduce neutrophil recruitment in LPS-induced airway neutrophilia (Ames et al., 2001). The C3a/C3aR interaction may be a candidate for therapy in asthma (Bautsch et al., 2000; Drouin et al., 2002). Antibodies neutralizing C3a have also been shown to abolish C3aR mediated function (Elsner et al., 1994). Disrupting the C3aR led to protective anti-inflammatory effects in endotoxin shock (Kildsgaard et al., 2000).

3.4.4. C5aR antagonists

The cyclic peptide AcF[OPdChaWR] (Finch et al., 1999) has been extensively studied in various animal models. It is active in both primates and rodents. Beneficial effects of this antagonist have been observed in I/R injury in small intestine and kidney (Arumugam et al., 2002, 2003) and in inflammatory bowel disease (Woodruff et al., 2003). It attenuated

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chemotaxis and cytokine production (Haynes et al., 2000), endotoxin-induced neutropenia (Short et al., 1999) and the reverse passive Arthus reaction (Strachan et al., 2000). It was effective when given orally to animals with immune-complex mediated dermal inflammation (Strachan et al., 2001) and experimental arthritis (Woodruff et al., 2002). This antagonist also exerted a protective role in anti-phospholipid syndrome with fetal loss (Girardi et al., 2003) and in hepatic I/R injury (Arumugam et al., 2004).

C5aR antagonists were also developed by screening of phage-display libraries and by site directed mutagenesis of C5a, both shown to be effective in reducing complement-mediated inflammation in vivo (Pellas et al., 1998; Heller et al., 1999). The dimeric recombinant human C5a antagonist, CGS 32359 (Pellas et al., 1998), attenuated neutrophil activation and reduced myocardial infarction size in a porcine model of surgical revascularization (Riley et al., 2000). Another C5aR antagonist significantly reduced murine renal I/R injury (deVries et al., 2003). A non-peptide C5aR antagonist active in cynomolgus monkeys and gerbils was shown to inhibit C5a mediated neutropenia after oral administration (Sumichika et al., 2002).

3.5. Other inhibitors

Numerous natural and synthetic substances have been documented to inhibit complement, but these are in general not complement specific. Examples are nafamostat mesilate (FUTHAN; FUT-175), aprotinin, K-76 monocarboxylic acid (MX-1) and heparin. The latter has a number of effects on complement and coagulation and modifications to obtain complement-inhibitory heparin without effect on coagulation have been made (Weiler et al., 1992). Dextran potentiates the effects several complement-inhibitory proteins including C1-inhibitor and was found to delay ex vivo hyperacute xenograft rejection (Fiorante et al., 2001). Coating of artificial devises with heparin improves biocompatibility by reducing complement activation and subsequent inflammatory reaction (Olsson et al., 2000).

High-dose intravenous immunoglobulins (IVIG) are currently used to treat various inflammatory diseases. The list of biological effects of IVIG is long, including several complement modulating activities. In fact, the effect of IVIG on complement is not by mediated by inhibition of complement activation. IVIG act as scavenger for C1q, C4b and C3b binding, thus diverting the activation products from the target to the immunoglobulin molecules (Frank et al., 2000). Thus, IVIG should not be used as a primary complement-inhibitor, but its effect on complement could be an additional benefit to certain inflammatory diseases.

Cobra venom factor (CVF) traditionally used for experimental complement depletion is frequently on the list of potential therapeutic complement-inhibitors. It should be noted, however, that CVF is not a complement-inhibitor, but a potent complement activator, which depletes C3 from plasma by activating the alternative pathway amplification loop. The activation per se may influence the results obtained, therefore, specific complement-inhibitors developed to date should replace CVF in animal studies.

4. Concluding remarks

Three decades ago the role of complement in human disease was largely unknown. Two decades ago, it became evident that complement activation was associated with a number of pathophysiologic conditions, although the role of this activation in the pathogenesis of the diseases was largely unknown. During the last decade studies using specific complement-inhibitors or complement knock-out animals have revealed that complement plays a crucial role in the pathogenesis of inflammatory tissue destruction. Based on these encouraging data, a great enthusiasm evolved for specific complement inhibition as a novel treatment of human diseases. Unfortunately, the progress towards a clinical application has been relatively slow. This may be attributed to several reasons: human diseases appear to be more complex in their pathophysiology than reflected by those induced in animal models. Experimental conditions differ significantly from those in the clinic with respect to time for and mode of application. The number of drugs which meanwhile reached the stage of clinical trials is markedly limited compared to those initially and still used in animal models. Finally, the selection of patients and size of test groups for a chosen indication may have been inadequate for a clear demonstration of a clinical benefit. There is still a need for more experimental studies to further elucidate the mechanisms of complement-mediated damage. This will provide a rational approach to design and optimise the treatment, i.e. identifying the component(s) needed to be inhibited in the actual condition and targeting the inhibition to the actual site of inflammation. The fate of this exciting new avenue in antiphlogistic therapy will depend not only on the development of novel complement-inhibitors, but also on the success of those which are already advanced to clinical trials.

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Current methods for the generation of human antibodies for the treatment of autoimmune diseases

Jane Osbourn, Lutz Jermutus and Alex Duncan

Autoimmune diseases are a significant area of unmet medical need in the Western World, but human antibodies are an emerging drug class that could address this demand. Some autoimmune diseases, such as rheumatoid arthritis, are currently benefiting from antibody treatment and new and existing technologies for antibody generation could facilitate the production of effective human antibodies as future drug candidates for other autoimmune diseases. Several methods of generating human antibodies for use as therapeutics have been established, the most commonly used being phage display and transgenic mouse technologies and more recently, cell-free display technologies have also emerged. In this review, we explain the principles behind the various methods of antibody generation and highlight some potential benefits of certain approaches in the context of treatment of autoimmune disease.

Jane Osbourn*
Lutz Jermutus
Alex Duncan
Cambridge Antibody Technology, Milstein Building Granta Park, Cambridge UK CB1 6GH
*e-mail: jane.osbourn@cambridgeantibody.com

The American Autoimmune Related Diseases Association (www.aarda.org) estimates that there are >80 autoimmune diseases, which, collectively, after heart disease and cancer, cause one third of all premature deaths and disability in the Western World. Among the most prevalent are Graves' Disease, rheumatoid arthritis (RA), Hashimoto's thyroiditis, multiple sclerosis (MS), Raynaud's syndrome, systemic lupus erythematosus (SLE), Sjogren's syndrome, scleroderma and Crohn's disease [1].

Disease mechanisms

The normal function of the immune system is to respond to invading microorganisms by producing antibodies or sensitized lymphocytes to recognize and destroy them. Autoimmune diseases occur when the normal function of the immune system is disrupted and antibodies to the patient's own cells and tissues are produced. These antibodies can interfere with cellular function and initiate tissue destruction, producing inflammation in the surrounding tissue or organ. The disease-producing processes in autoimmunity are referred to as hypersensitive reactions, which are similar to those observed in allergy, except that in autoimmune disorders, the hypersensitivity response is to the body itself, rather than to an outside substance.

Autoimmune disorders are classified into organ-specific and non-organ-specific types. In organ-specific types, the reaction is directed against one particular organ – examples include Hashimoto's thyroiditis (thyroid) and Addison's disease (adrenal glands). In non-organ-specific disorders, the effects are widespread throughout the body, for example SLE and RA. Most autoimmune diseases are chronic, requiring lifelong care and monitoring, and no cure for any of these diseases has yet been developed.

Current treatments

In autoimmune disorders, such as RA, established treatments relieve pain and inflammation, or suppress the immune response. Pain-relief drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids reduce inflammation but are also associated with a wide range of side-effects. Commonly used NSAIDs, such as the cyclooxygenase 2 (COX-2) inhibitors, can cause a variety of problems, including gastrointestinal toxicity, which results in abdominal pain, nausea, indigestion and ulcers [2,3]. Corticosteroids, such as prednisone, are highly effective at relieving pain but can cause weight gain [4] and, in some patients, can contribute to the development of osteoporosis [5].

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Alternative RA treatments, aimed at slowing joint destruction, include a range of widely used disease-modifying anti-rheumatic drugs (DMARDs), such as methotrexate and chlorquine. Although effective, these drugs must be taken for several months before they show any positive effects. They are also non-specific to the site of inflammation and therefore, cause side effects ranging from abdominal discomfort, rashes, gastrointestinal toxicity and nausea to decreases in platelets, or white and red blood cell counts [6].

The variety of severe side effects associated with RA treatments has prompted a move towards the development of drugs that directly target the causes of the disease rather than attempting to alleviate the symptoms. For RA treatment, these 'biological response modifiers' (BRMs) directly target the cytokines, such as tumour necrosis factor (TNF-α), that are involved in the development and progression of the disease. Treatments include Enbrel®, a TNF-α receptor protein [7], and the antibody drugs Remicade® [8] and HUMIRA™ [9], which bind specifically to TNF-α. In clinical trials, these BRMs have demonstrated efficacy in reducing the symptoms of RA and in inhibiting joint erosion. Antibodies are particularly suited to the role of a BRM, because they can bind specifically and with high affinity to a wide range of biological molecules and, because they have a longer half-life than small-molecule treatments.

Monoclonal antibodies

The production of hybridoma cell lines is perhaps the most established technique for generating monoclonal antibodies [10]. Hybridomas are generated by immunizing a mouse with a target antigen, then fusing the antibody-producing B cells of that mouse with mouse tumour cells. The hybridoma possesses the immortal growth properties of the tumour cell and thus, can be cultured indefinitely, and can secrete antibody with the specificity of the original B cell. The homogenicity and specificity of monoclonal antibodies that are produced from hybridomas makes them particularly suitable for *in vivo* administration for therapeutic purposes. However, clinical studies using mouse monoclonal antibodies have been disappointing, because the human immune system recognizes the mouse antibodies as 'foreign'. This results in rapid clearance of the mouse protein by the immune system of the patient and, in some cases, can lead to the induction of a severe allergic reaction to the mouse antibodies – an effect known as the human anti-mouse antibody (HAMA) response.

Chimaeric monoclonal antibodies

To reduce the potential for HAMA responses, variable regions from mouse antibody genes can be recombined with constant regions from human antibody genes. The recombinant gene encodes a chimaeric mouse–human antibody that has antigenic specificity derived from the mouse, but a human isotype. Hence, the resultant protein has human effector functions and fewer mouse antigenic determinants, and is therefore less likely to be immunogenic in humans. Mouse variable regions can also cause an immune response in humans, and chimaeric antibodies containing only mouse complementarity determining regions (CDRs) have subsequently been developed. CDRs are the regions of the antibody molecule that are largely responsible for antibody–antigen binding and including only these elements rather than the full variable region of the antibody, further reduces the risk of immunogenicity. Replacing some mouse CDR sequences with human sequences can further humanize antibodies; mouse residues are minimized but retained at the key binding regions, to maintain affinity.

By the start of 2003, nine monoclonal antibodies had been granted a biologics licence approval (BLA) by the US Food and Drug Administration (FDA) (www.fda.gov) and all of these were of the chimaeric mouse–human or humanized type. These antibodies are being successfully used to treat a variety of conditions, including leukaemia, organ rejection, non-Hodgkin's lymphoma and breast cancer. In addition, one of these chimaeric antibodies, Remicade®, is approved for the treatment of the autoimmune disorders, RA and Crohn's disease, and there are also several other chimaeric or humanized antibodies currently in clinical trials for the treatment of a range of autoimmune diseases (Table 1).

Many chimaeric antibodies still cause adverse events, such as headache, fever, chills [11] and respiratory infection [12] and, in some patients, these events can be associated with allergic reactions to the drug. One solution to some of the immunogenic responses induced by chimaeric antibody drugs is to develop human antibody therapeutics, which contain no mouse-derived sequences. Transgenic mice and phage display techniques are the key technologies that have been widely used to isolate such human antibodies.

Transgenic mice

Transgenic mouse technology for antibody drug discovery involves the introduction of human antibody genes into the mouse genome [13,14]. The mice are engineered to suppress their own antibody production and large sections of the human antibody heavy and light chain loci are then transferred into these animals; the human antibody genes are functional in the context of the mouse machinery for antibody recombination and expression. The mouse antibody-

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Table 1. Chimaeric and humanized antibodies developed for the treatment of autoimmune diseases

Product name	Generic name	Antibody type	Indication	Clinical development phase	Company
Remicade®	Infliximab	Anti-TNF-α	Crohn's Disease, RA, psoriasis	FDA approved	Centocor
Campath®	Alemtuzumab	Anti-CD52	MS	FDA approved	BTG, ILEX, Millennium, Schering AG
Raptiva™	Efalizumab	Anti-CD11	Psoriasis	Phase III BLA filed	Xoma, Serono, Genentech
Antegren®	Natalizumab	Anti-α_4 integrin	MS	Phase III	Elan, Biogen, Genzyme
Humicade™	CDP 571	Anti-TNF-α	Crohn's Disease	Phase III	Celltech, Biogen, Genzyme
CDP 870	CDP 870	Anti-TNF-α	RA and Crohn's Disease	Phase III	Celltech, Pharmacia
Antegren®	Natalizumab	Anti-α_4 integrin	MS, Crohn's Disease	Phase III	Elan, Biogen, Genzyme
MLN02	MLN02	Anti-$\alpha_4\beta_7$ integrin	Crohn's Disease	Phase II	Millennium, Genentech
HuZAF	Fontolizumab	Anti-interferon-γ	Crohn's Disease, psoriasis	Phase II	PDL
IDEC-151	Clenoliximab	Anti-CD4	RA	Phase II	IDEC
Zenapax®	Daclizumab	Anti-IL-2	Uveitis	Phase II	PDL
5G1.1	Eculizumab	Anti-C5	RA	Phase II	Alexion
MEDI-507	Siplizumab	CD2 agonist	Psoriasis	Phase II	Biotransplant, MedImmune

Abbreviations: IL, interleukin; MS, multiple sclerosis; RA, rheumatoid arthritis; TNF, tumour necrosis factor.

producing B cells can then be used to generate hybridoma cell lines for human monoclonal antibody production. In the usual time span required for hybridoma generation and characterization, a transgenic mouse can produce a range of specific antibodies from which potential drug candidates can be identified. If further optimization of the antibody is required, the genomic information that encodes the antibody of interest must be cloned and alternative antibody engineering approaches employed.

Companies using this approach include Abgenix (http://www.abgenix.com) and Medarex (http://www.medarex.com). Medarex has licensed its technology to Genmab A/S (http://www.genmab.com) and also collaborates with Kirin Brewery (http://www.kirin.co.jp/). These companies have taken antibody drugs into various stages of clinical trials, with Medarex having two antibodies to treat different autoimmune diseases in mid-stage clinical trials (Table 2). No significant allergic reactions have been noted so far and thus, the safety profiles seem better than with chimaeric antibodies.

Phage display

The other established method of developing human monoclonal antibodies is phage display technology. This

Table 2. Human antibodies in development to treat autoimmune diseases

Product name	Generic name	Antibody type	Indication	Method of generation	Clinical development phase	Company
HUMIRA™	Adalimumab	Anti-TNF-α	RA	Phage display	FDA approved	CAT, Abbott
HuMax-CD4		Anti-CD4	Psoriasis	Transgenic mice	Phase II	Genmab, EISAI
HuMax-IL15		Anti-IL-15	RA, psoriasis	Transgenic mice	Phase II	Genmab, Medarex
HUMIRA™	Adalimumab	Anti-TNF-α	Juvenile RA, Crohn's Disease	Phage display	Phase II	CAT, Abbott
CAT-192	Metelimumab	Anti-TGF-β	Scleroderma	Phage display	Phase II	CAT, Genzyme
ABT-874		Anti-IL-12	Crohn's disease, RA	Phage display	Phase II	CAT, Abbott, Wyeth Research

Abbreviations: IL, interleukin; MS, multiple sclerosis; RA, rheumatoid arthritis; TGF, tissue growth factor.

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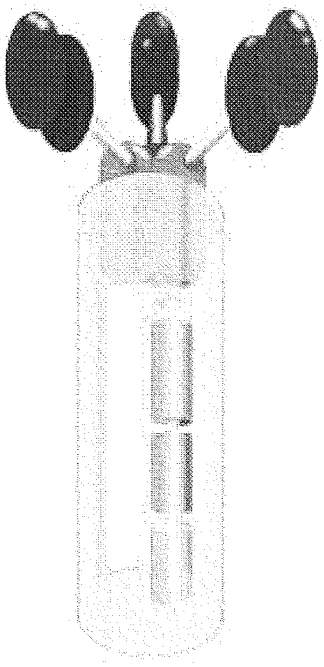

Figure 1. A filamentous phage displaying an antibody protein at its tip. An antibody variable region sequence is inserted into the phage genome linked to the gene 3 coat-protein sequence. The antibody sequence is then 'displayed' as a gene 3 fusion protein on the outside of the phage. The key feature of phage display is that the phenotype and genotype are linked in a single molecule.

technique, as first described for antibodies in 1990 [15], exploits the ability of filamentous bacteriophage, such as M13, to express foreign proteins on the phage surface (Figure 1), enabling the gene and gene product to be physically linked.

Phage antibody libraries are produced by cloning variable regions of human antibody genes, derived from naïve, immunized or synthetic antibody repertoires, into the phage coat-protein genes of phagemid vectors. These libraries are introduced into *Escherichia coli* cells, which are then superinfected with helper phage, to facilitate the replication and assembly of viable phage particles. Phage libraries contain a huge variety of antibody variable region sequences [16] and some libraries now contain $>10^{11}$ unique phage antibodies. Each phage particle in the library encapsidates the DNA that encodes the antibody expressed on its surface and antibodies specific to a defined target can be selected from these large phage libraries by allowing the library to bind to immobilized antigen – phage antibodies that bind to the antigen are thereby captured and non-binding phage washed away. Antibody variable regions selected using this technique can be reformatted into IgG molecules by combination with human antibody constant regions and subsequently expressed in mammalian cell lines.

The great advantage of phage display is that once a library has been created, it can be used to select for antibodies that bind to any target antigen of interest, with the selection and primary screening process being completed in only 2–3 weeks.

Selection from the library can also be tailored to produce a range of output populations, depending on the desired characteristics of the antibody drug. For example, it might be necessary to generate an antibody with the ability to bind to a specific epitope on the target molecule, the ability to cross-react with a rodent antigen, or the ability to recognize a given cell type to name but a few. When an antibody with the desired characteristics has been isolated, the phage system also provides the format for the engineering of antibody affinity or potency. The diversity of antibody populations generated using phage display means that antibodies that bind to different parts of the same target antigen molecule can be isolated. This also makes the technique highly applicable to functional genomics, because it can rapidly generate lead candidate antibodies to large numbers of proteins in high throughput.

Several biotechnology companies use phage display technology to produce human antibody therapeutics, including Cambridge Antibody Technology (CAT, http://www.cambridgeantibody.com), Crucell (http://www.crucell.com), Dyax (http://www.dyax.com) and MorphoSys (http://www.morphosys.de/). The world's first human antibody to treat RA, HUMIRA™, is now on the market. This antibody was isolated and optimized by CAT in collaboration with Abbott Laboratories (http://abbott.com). All the human antibodies that are currently in clinical trials (from Phase II) for autoimmune diseases are listed in Table 2.

HUMIRA™ demonstrates the effectiveness of human monoclonal antibodies derived from phage display. Published data from a study of 271 patients showed that HUMIRA™ improved the symptoms of RA in up to 50% of patients. In addition, only minor side effects, such as injection-site reactions, rashes and headaches were reported, suggesting that the drug is safe and well tolerated at the doses used [9].

Abbott submitted information from this trial and several others to the FDA and European Agency for the Evaluation of Medicinal Products (EMEA, http://www.emea.eu.int/), obtaining marketing approval from the FDA on 31 December 2002. This made HUMIRA™ the first human monoclonal antibody therapy on the market for the treatment of an autoimmune disease.

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Emerging *in vitro* display technologies for creating human antibodies

Transgenic mice and phage display are both cell-based development technologies, and hence, they are limited by the physical constraints of getting DNA into a cellular host. To overcome this restriction, two new cell-free translation technologies have been developed, known as 'covalent display' and 'ribosome display'. These techniques promise the rapid construction of display libraries, containing up to 10^{14} molecules – significantly larger than phage display libraries – and thus, provide an increased probability that the desired specificity and potency required in the therapeutic candidate can be directly selected.

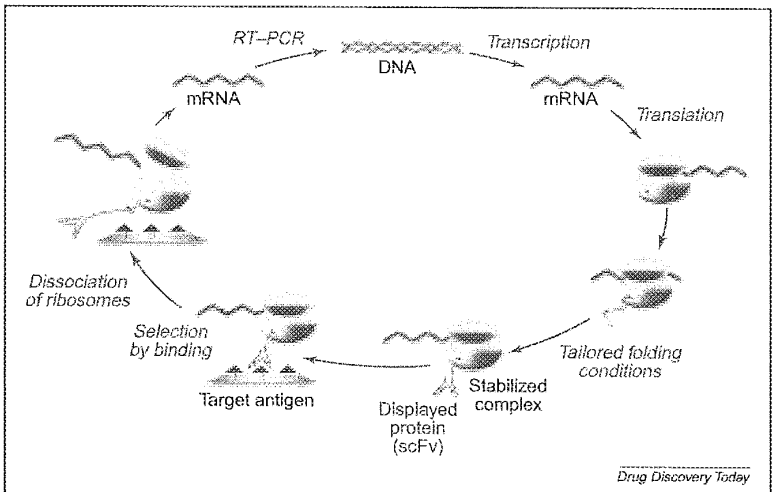

Figure 2. The ribosome display process. This cell-free process, analogous to phage display, involves the following steps: i) *in vitro* transcription of an antibody DNA library; ii) *in vitro* translation of mRNA molecules that contain a short spacer sequence and lack a stop codon; iii) folding of antibody molecule while tethered to the ribosome and encoding mRNA; iv) selection of antibody–ribosome–mRNA complexes by binding to immobilized target antigen (any non-binding antibodies are washed away); v) release of selected mRNA molecules by alteration of wash conditions and; vi) generation of DNA by RT–PCR for use in further rounds of selection.

Ribosome display

Ribosome display works by exploiting the cellular role of ribosomes to synthesize proteins. Ribosomes do this by binding to mRNA and travelling along the molecule, reading the nucleotide sequence and assembling a chain of amino acids as they move. In the cell, the fully formed protein is then released and the mRNA dissociates from the ribosome. The ribosome display system uses mRNA encoding a repertoire of antibody genes in a cell-free expression system (either *E. coli* or eukaryotic cell lysate), so enabling assembly of antibody molecules *in vitro*.

A short mRNA spacer is included at the beginning of the antibody sequence to ensure that the antibody molecule is fully extruded from the ribosomal tunnel. Cellular mRNA molecules have a start and stop codon on either side of the protein coding sequence; in the ribosome display system, the stop codon at the end of the antibody-coding region is removed, preventing the release of the newly translated antibody protein. The antibody remains tethered to the ribosome, which, in turn, remains associated with the corresponding antibody gene in the form of mRNA (Figure 2). Conditions in the cell-free lysate environment can be tailored to ensure the correct folding of the antibody molecule.

The ribosome display selection process is analogous to that of phage display, with antibody–ribosome– mRNA complexes being selected by binding to immobilized target antigen and any non-binding antibodies being washed away. The selected mRNA can be released from the ribosomes by alteration of the salt concentration of the wash buffer. RT–PCR can then be performed to generate a population of selected DNA that can be used as the starting point for further rounds of selection

Using ribosome display, single-chain antibodies specific for a yeast transcription factor GCN4 have been selected [17]. In addition, ribosome display has been successfully used to generate anti-progesterone antibody fragments [18]. There is also evidence that ribosome display can produce variable domain (CTLA-4) libraries and could be used to provide specific targeting to either novel or refractory cancer markers [19]. Ribosome display technology (acquired and developed for commercial purposes by CAT) has the potential to become a versatile tool for the development of human monoclonal antibodies [20] with picomolar affinities [21].

The main advantage of this technique is that it has the potential to generate larger libraries of antibodies than phage display, hence producing still more diverse selectable populations, while offering many of the same benefits of a phage display library. These include the ability to produce and screen the library for binding activities in a matter of weeks and the provision of a format for the directed evolution of antibodies to improve desirable characteristics, such as affinity, stability and potency [22].

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Covalent display

Other emerging display technologies, known as covalent display, have been developed by Isogenica [23] (http:// www.isogenica.com) and Phylos [24] (http://www.phylos.com).

The Phylos PROfusion™ mRNA display system relies upon puromycin-mediated covalent coupling of protein to the RNA that encodes it [25], and is conducted entirely as isolated biochemical reactions, outside of the host cells. The process begins with oligonucleotide synthesis to construct a population of DNA molecules, each containing defined regions with signals for RNA transcription, protein translation, purification tags and PCR amplification. The molecules also have regions of diversity, which encode for a protein scaffold and a unique protein-binding site [26].

The library DNA is transcribed to generate RNA and a peptide acceptor is covalently attached to the 3′ end of mRNA, using a synthetic linker. During *in vitro* translation in a cellular lysate, the ribosome moves along the mRNA to generate a protein.

When the ribosome has reached the end of the encoding mRNA, it pauses, allowing time for the peptide acceptor to covalently link to the C-terminus of the protein. The result is a collection of up to 10^{13} possible binding proteins, each chemically linked to the RNA that encodes it.

The molecules can then be purified from the reaction mixture using target proteins that are captured on magnetic beads. The beads are washed to remove nonspecific or weakly binding molecules and the nucleic acid of the PROfusion™ molecules that bind to the target molecule are recovered by PCR, creating a new pool of nucleic acids enriched with high-affinity binders for the target of interest.

The Isogenica system uses a replication initiator protein (P2A) from the *E. coli* phage, P2, to initiate rolling circle replication by binding to the viral origin and introducing a nick in the DNA. This exposes the 3′-OH group, which then initiates DNA synthesis [27–29]. Nicking also exposes a 5′ phosphate, which attaches to a tyrosine residue in the active site of P2A, thus binding the P2A to the DNA molecule from which it has been expressed. This enables pools of polypeptides that are fused to P2A to be synthesized *in vitro*, so that they become attached to their own coding sequence and can be selected on an immobilized antigen.

Recently, the Phylos PROfusion™ mRNA display system has been used to produce protein microarrays [30]. However, neither of the technologies described has, to date, generated a therapeutic antibody, although development is ongoing.

Summary and prospects

One chimaeric monoclonal antibody (Remicade®) has been FDA-approved as a treatment for RA and Crohn's disease and 11 are in mid–late-stage clinical trials; to date, chimaeric or humanized antibodies are the most commercially advanced types of antibody therapeutics for the treatment of autoimmune diseases. This is due partly to the fact that methods for generating chimaeric antibodies have been available for longer than the alternative technologies and have, therefore, produced more antibodies for clinical development. Many of these antibodies have significant side effects relating to their immunogenicity in human patients.

With the approval of HUMIRA™ (the first human antibody to treat RA) and an additional four human antibodies in Phase II or III trials for the treatment of autoimmune diseases, transgenic mice and phage display systems are now proving effective in the field of drug development. The benefit of these technologies is the ability to generate wholly human antibodies, which do not produce the immunogenic side effects that are seen with infusions of chimaeric antibodies. Short lead-generation times for antibodies made by phage display or transgenic mouse technology and, in the case of phage display, the ability to further optimize these lead candidates, makes the possibility of high potency therapeutics from these methods a reality.

Owing to their relatively recent emergence, neither covalent nor ribosome display has so far produced any human antibodies for use as clinical candidates in autoimmune disease. However, both technologies can produce libraries that are potentially larger than either phage display or chemical libraries, and so they could, in the next five years, become more widely adopted. Successful technical developments already demonstrated within the ribosome display system could mean that these technologies become the systems of choice for the generation and optimization of human antibodies against new drug targets for autoimmune diseases in the near future.

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Other News To Note

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Abstract (English): Accelrys Inc., of San Diego, reported the availability of five Accelrys software products on IBM eServer pSeries systems running AIX. These are the first product releases stemming from the global strategic alliance between IBM and Accelrys. The applications are geared toward drug discovery and bioinformatics.
Alexion Pharmaceuticals Inc., of Cheshire, Conn., completed enrollment in its first Phase II trial in patients with a severe form of kidney disease, known as membranous nephritis, who are being treated with its anti-inflammatory C5 inhibitor antibody, 5G1.1, now called eculizumab. Separately, Alexion received orphan drug designation from the FDA for the use of eculizumab for this disease. The Phase II multicenter, double-blinded, randomized, placebo-controlled study examines the safety and efficacy of repeated doses of eculizumab over four months.

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Alkermes Inc., of Cambridge, Mass., signed an agreement with Indianapolis-based Eli Lilly &Co. that provides for an investment by Lilly in AlkermesE commercial-scale production facility for inhaled pharmaceutical products based on AlkermesE AIR pulmonary drug delivery technology. The facility will have the capability to produce multiple products and is under construction in Chelsea, Mass. Alkermes and Lilly are collaborating on the development of inhaled formulations of insulin for diabetes. Lilly will make an investment in the Chelsea facility to fund pulmonary insulin production and packaging capabilities. If inhaled formulations of insulin are developed successfully and are commercially launched, Lilly will purchase product from the facility. (See BioWorld Today, April 3, 2001.)
Amarin Corp. plc, of London, said its subsidiary, Amarin Development AB, of Malmo, Sweden, will establish a strategic technology partnership with NanoCarrier Co. Ltd., of Chiba, Japan. The partnershipEs goal is to develop controlled-release technologies for use in pharmaceutical products, and the companies will share the costs of development together with any resulting commercial benefits. Initially, the collaboration will focus on the feasibility of developing a technology for the oral controlled release of nanoparticles, using AmarinEs Diffusion Controlled Vesicle controlled-release technology and NanoCarrierEs micellar nanoparticle controlled-release technology.
Amylin Pharmaceuticals Inc., of San Diego, reported the exercise by its underwriters of an overallotment option to purchase 1.57 million shares of common stock associated with its follow-on stock offering that priced on Feb. 11. Including the overallotment shares, the offering totaled 12 million shares at $8 per share, resulting in expected net proceeds to the company of about $91 million. The joint lead managers for the offering were Goldman Sachs and Lehman Brothers. (See BioWorld Today, Jan. 31, 2002, and Feb. 13, 2002.)
Applied Genetic Technologies Corp., of Alachua, Fla., completed a license agreement with Johns Hopkins

University in Baltimore for use of adeno-associated virus gene therapy vectors in muscle tissue. The agreement gives AGTC the right to develop the AAV technology for injection into human muscle tissue for potential therapy of alpha-l antitrypsin deficiency and PompeEs disease.

AtheroGenics Inc., of Atlanta, completed a Phase I trial demonstrating the safety of AGIX-4207, its lead oral compound for rheumatoid arthritis. The results in 48 healthy volunteers indicated that it was well tolerated over the single and multiple dose ranges studied. AtheroGenics plans to initiate a Phase II efficacy study in patients with rheumatoid arthritis in the second quarter.

BioMicro Systems Inc., of Salt Lake City, entered agreements with the National Cancer Institute, the Huntsman Cancer Institute and the Buck Institute for Age Research to evaluate its Microarray User Interface (MAUI) hybridization product. The alpha sites will test BioMicroEs MAUI microfluid analysis product used to expedite the drug discovery process by obtaining results from microarray hybridization.

Atugen AG, of Berlin, signed a technology evaluation agreement with Kinetek Pharmaceuticals Inc., of Vancouver, British Columbia, which focuses on the discovery and development of selective signal transduction inhibitors in cancer and inflammatory diseases. Kinetek will evaluate the application of AtugenEs target-validation technology in its research and development efforts in oncology. Atugen will optimize its GeneBloc and cellular delivery technologies in specific cell lines to knock down the expression of a number of signal transduction kinase targets identified by Kinetek. Subsequently, Kinetek will use the optimized GeneBloc molecules to validate the gene function of these targets using its proteomics technologies. Financial details were not disclosed.

Bayer AG, of Leverkusen, Germany, and Aventis Pharma SA, of Antony, France, signed a nonbinding letter of intent to establish a joint venture for biological products. The companies plan to combine BayerEs Biological Products Business Group with AventisE subsidiary, Aventis Behring. Bayer will own a majority interest and have operational control. Included are the marketing rights to factor VIII products that will continue to be manufactured by Bayer using genetic engineering techniques. Bayer will have the option to acquire the remaining interest at a later date. BayerEs biological group, headquartered in Research Triangle Park, N.C., has products that are derived from blood plasma. Aventis Behring focuses its research and development efforts on proteins from human blood plasma.

BioSeek Inc., of Burlingame, Calif., raised $8.4 million in its first round of venture financing. The Series A round investors included Bay City Capital, Fremont Ventures, SImile Investors LLC and Vanguard Ventures. BioSeekEs president and CEO said the money will allow it to aggressively develop and exploit its platforms for functional genomics and drug discovery.

Chiron Corp., of Emeryville, Calif., completed a cash tender offer by Manon Acquisition Corp., a wholly owned subsidiary of Chiron Corp., for all the outstanding common stock of Matrix Pharmaceutical Inc., of Fremont, Calif. The tender offer expired as scheduled on Tuesday. Based on preliminary information, a total of 22.7 million shares representing about 86 percent of the outstanding common stock of Matrix were validly tendered, Chiron said. Chiron, Manon and Matrix entered into a merger agreement pursuant to which Manon began on Jan. 14 an all-cash tender offer for all of MatrixEs outstanding common stock at $2.21 per share. (See BioWorld Today, Jan. 8, 2002.)

ChromaVision Medical Systems Inc., of San Juan Capistrano, Calif., entered an agreement with Abbott Laboratories, of Abbott Park, Ill., to support the clinical development of AbbottEs cancer compounds targeting tumor angiogenesis. ChromaVisionEs imaging solution technology, Automated Cellular Imaging System, rare-event detection capability and ChromaVision-labeled antibodies and reagents will be employed in the research and assessment of the efficacy of AbbottEs developmental anti-angiogenic compounds.

Ciphergen Biosystems Inc., of Fremont, Calif., said it sold multiple ProteinChips systems to hospitals, universities and government agencies in China, which intend to focus their initial use of the systems on protein expression profiling in a variety of diseases to develop predictive diagnostic tests. Ciphergen also established a

formal relationship with the Universities Confederated Institute for Proteomics, established by the Chinese government in May 2001. The company also agreed to provide priority technical assistance to UCIP members, which include many of the medical colleges and universities in China.

CombiMatrix Corp., of Mukilteo, Wash., a majority-owned subsidiary of Acacia Research Corp., was awarded a Phase I National Institutes of Health grant for the development of its protein biochip technology. The title of the grant is oSelf-Assembling Protein Microchips.o The grant is in addition to a three-year Phase I and a Phase II Small Business Innovation Research grant from the U.S. Department of Defense for the development of multiplexed chip-based assays for chemical and biological warfare agent detection.

EraGen Biosciences Inc., of Madison, Wis., named Irene Hrusovsky president and CEO and appointed her to the board. She is a former executive of GlaxoSmithKline plc, of London, where she served as vice president, general medicine/medical marketing strategy, clinical operations. Prior to her position at GSK, Hrusovsky spent more than 20 years at Abbott Laboratories, of Abbott Park, Ill. EraGen licenses bioinformatics, drug screening platforms, nucleic acid and genotyping technologies for clinical diagnostic and life science research applications.

Illumina Inc., of San Diego, signed an agreement with Amersham Biosciences, the life sciences business of Amersham plc, of London, granting Illumina a nonexclusive worldwide license under five fundamental patents involving engineering designs and methods for confocal scanning for use with its BeadArray product line. Additional terms of the agreement were not disclosed.

ImmunoGen Inc., of Cambridge, Mass., and Millennium Pharmaceuticals Inc., of Cambridge, Mass., signed an exclusive product license for use of ImmunoGenEs maytansinoid tumor-activated prodrug (TAP) technology with MillenniumEs MLN591 antibody. This triggers MillenniumEs payment of a product-licensing fee to ImmunoGen. In March 2001, the companies announced the formation of a collaboration that provides Millennium with access to ImmunoGenEs TAP technology and enables Millennium to obtain exclusive product licenses for a restricted number of specific antigen targets. MLN591 is directed toward the extracellular domain of the prostate-specific membrane antigen. ImmunoGenEs TAP technology couples the companyEs anticancer agents with tumor-targeting antibodies to achieve antibody-directed killing of cancer cells. (See BioWorld Today, March 7, 2001.)

Molecular Diagnostics Inc., of Chicago, and Esoterix Inc., of Austin, Texas, said Esoterix will use Molecular DiagnosticsE In-Cell HPV probe for an upcoming clinical trial of new approaches to identify patients with cervical cancer. The In-Cell HPV probes are designed to detect messenger RNA of the E6 and E7 oncogenes, and provide an alternative to HPV DNA testing to screen women with HPV infections.

ProteoTech Inc., of Kirkland, Wash., received funding totaling $2.78 million from the National Institute of Aging for two research programs. The programs are designed to lead to therapeutics for the prevention and treatment of brain amyloid protein deposits present in patients with AlzheimerEs disease. It also is designed to create a closer understanding of the mechanisms involved in AlzheimerEs amyloid brain plaque formation and persistence.

Samaritan Pharmaceuticals Inc., of Las Vegas, said that Georgetown University in Washington notified Samaritan of the discovery of a potential therapeutic for AlzheimerEs disease, and Samaritan licensed the product. This is the first discovery to result from the collaboration. Samaritan initiated a seven-year contract to fund Samaritan Research Laboratories at Georgetown in July 2001.

Synthetic Blood International Inc., of Costa Mesa, Calif., said that company officials are planning to meet with the FDA before the end of April to review plans to file an investigational new drug application for Phase I clinical trials with Oxycyte. The company said it expects the IND to be filed by the end of the year. Oxycyte, a blood substitute, is one of two perfluorocarbon-based products being developed by the company.

Synthon Chiragenics Corp., of Monmouth Junction, N.J., introduced DiscoveryCollections, a program that makes SynthonEs chemistry platforms available to companies for independent research. Four collections

Diamond, Platinum, Gold and Silver will consist of palettes of compounds representing different aspects of a technology platform for drug discovery.

Target Discovery Inc., of Palo Alto, Calif., raised more than $4.6 million in Series A financing for its proteomics platform technologies for comparison and identification of differences in the protein makeup of healthy and diseased tissues. Clayton Struve, its largest investor, was joined by other private angel investors from the Chicago area.

U.S. Genomics Inc., of Woburn, Mass., established a research collaboration with Pui-Yan Kwok, a researcher focused on genetics and technology at Washington University School of Medicine in St. Louis. KwokEs laboratory will employ U.S. GenomicsE direct, linear DNA analysis technology for multiple applications. Kwok also will work to extend the development of the technology platform toward additional applications in the field of genetics. Financial terms were not disclosed.

Xencor Inc., of Monrovia, Calif., signed licensing agreements with the California Institute of Technology and Pennsylvania State University to further expand its protein design automation platform. The agreements expand the patent estate of the PDA technology and increase XencorEs computational throughput and accuracy in protein optimization. Xencor said the PDA technology is the first and only protein optimization method to combine advanced computational methods, high-performance computing and experimental screening.

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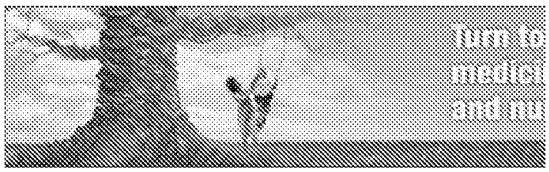

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Research Article

Requirement of activation of complement C3 and C5 for antiphospholipid antibody–mediated thrombophilia

Silvia S. Pierangeli , Guillermina Girardi, Mariano Vega-Ostertag, Xiaowei Liu, Ricardo G. Espinola, Jane Salmon

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Abstract

Objective

Antiphospholipid antibodies (aPL) have been shown to induce thrombosis, activate endothelial cells, and induce fetal loss. The pathogenesis of aPL-induced thrombosis, although not completely understood, may involve platelet and endothelial cell activation as well as procoagulant effects of aPL directly on clotting pathway components. Recent studies have shown that uncontrolled complement activation leads to fetal death in aPL-treated mice. In this study, we tested the

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hypothesis that aPL are responsible for activation of complement, thus generating split products that induce thrombosis.

Methods

To study thrombus dynamics and adhesion of leukocytes we used in vivo murine models of thrombosis and microcirculation, in which injections of aPL were used.

Results

Mice deficient in complement components C3 and C5 were resistant to the enhanced thrombosis and endothelial cell activation that was induced by aPL. Furthermore, inhibition of C5 activation using anti-C5 monoclonal antibodies prevented thrombophilia induced by aPL.

Conclusion

These data show that complement activation mediates 2 important effectors of aPL, induction of thrombosis and activation of endothelial cells.

Antiphospholipid syndrome (APS) is characterized by increased risk of vascular thrombosis, involving the venous, arterial, and placental circulatory systems. The pathogenic mechanisms for antiphospholipid antibody (aPL)–induced thrombosis are incompletely understood. Passive transfer of IgG from aPL-positive sera (IgG-APS) has been found to induce fetal loss, thrombosis, and endothelial cell activation in mice, suggesting a direct pathogenic role of aPL (1–3). Complement activation is a necessary intermediary event in the pathogenesis of fetal loss associated with aPL in this model (4, 5).

It is well established that activated complement fragments themselves have the capacity to bind and activate inflammatory and endothelial cells, as well as induce a prothrombotic phenotype either directly through C5b–9 (membrane attack complex [MAC]) or through C5a receptor (C5aR)–mediated effects (6, 7). Endothelial cells can release tissue factor in response to C5a activation (8). Inflammatory cells, when triggered by complement proteolytic products C5a and C3a, respond with the production of selected procoagulant activities, thereby initiating the coagulation pathways. MAC has also been associated with thrombosis. Studies performed in rats showed that CD59, an inhibitor of C5b–9 assembly and insertion, serves a protective role in a rat model of thrombotic microangiopathy, demonstrating that C5b–9 plays a critical role in the pathogenesis of thrombosis (9).

In previous studies, we demonstrated that the complement C3 convertase inhibitor, Crry, inhibited IgG -APS–induced thrombosis, suggesting that complement activation is required in IgG-APS–induced thrombophilia (4). Moreover, Girardi and coworkers proposed that heparin, the current standard treatment in patients with APS, prevents obstetric complications by blocking activation of complement, as opposed to preventing placental thrombosis (10). We therefore tested the hypothesis that complement activation mediates endothelial cell activation and the thrombogenic effects of IgG-APS.

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MATERIALS AND METHODS

Mice.

C3-deficient mice were obtained from V. M. Holers (University of Colorado Health Sciences, Denver) (11). The mice were generated by intercrossing $C3^{+/-}$ mice at F1 during a backcross to C57BL/6 and then propagating $C3^{-/-}$ progeny. $C5^{-/-}$ mice (B10.D2-$H2^{d}$H2-T18^{c} Hc^{0}/o2Sn) and the $C5^{+/+}$ background strain of mice (B10.D2-$H2^{d}$H2-T18^{c} Hc^{0}/oSnJ) were obtained from The Jackson Laboratories (Bar Harbor, ME). CD1 male mice (weight ~30 grams) were obtained from Charles River Laboratories (Wilmington, DE). All animals were housed in the animal care (American Association of Laboratory Animal Care–approved) facilities of the Morehouse School of Medicine. Animals were handled by trained personnel according to Institutional Animal Care and Use Committee guidelines.

Preparation of IgG-APS.

Antiphospholipid antibodies from APS patients (IgG-APS) were affinity-purified using cardiolipin liposomes and protein G–Sepharose chromatography as previously described (2, 3). Human IgG from a non-autoimmune healthy individual (IgG-NHS) was purified by an identical method. The sterile-filtered IgG fractions were determined to be free of endotoxin contamination by the limulus amoebocyte lysate assay (E-Toxate; Sigma, St. Louis, MO). Protein concentration was determined using the method of Lowry (12). Levels of human anticardiolipin (aCL) and anti–β_2-glycoprotein I antibodies were measured by standard enzyme-linked immunosorbent assay, performed as previously described (2, 3).

Analysis of thrombus dynamics and leukocyte adhesion in complement-deficient mice.

To investigate the role of C3 and C5 in thrombophilia induced by aPL, $C3^{+/+}$ and $C3^{-/-}$ mice (n = 7–10 animals) and $C5^{+/+}$ and $C5^{-/-}$ mice (n = 7–10 animals) were injected intraperitoneally with 500 μg of IgG-APS or with 500 μg of IgG-NHS twice, at time 0 and at 48 hours later. Surgical procedures to study thrombus dynamics and adhesion of leukocytes to the endothelium of postcapillary venules (number of adhering white blood cells [WBCs]) in the exposed cremaster muscle were performed 72 hours after the first IgG-APS or IgG-NHS injection, as described previously (2, 3). The mouse model of thrombosis formation used in this study has previously been described in detail (2, 3). Briefly, mice were anesthetized and the right femoral vein was exposed, resulting in a 0.5-cm segment of vein free for manipulation and observation. The vein was pinched with a pressure of 1,500 gm/mm^2 to introduce a standardized injury that induced a clot. Clot formation and dissolution in the transilluminated vein were visualized with a microscope equipped with a closed-circuit video system (including a color monitor and a recorder). Thrombus size (expressed in μm^2) was measured 1 minute after the pinch injury by freezing the digitized image and tracing the outer margin of the thrombus. Three-to-five thrombi were successfully induced in each animal, and mean values were computed.

Adhesion of leukocytes to endothelium in the cremaster muscle is an indication of endothelial cell activation (3). The number of leukocytes (WBCs) adhering within 5 different venules in the cremaster

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muscle was determined, and adhesion was defined as the presence of WBCs that remained stationary for at least 30 seconds (2, 3).

Analysis of thrombus dynamics using the anti-C5 monoclonal antibody (mAb).

Anti-C5 mAb have been shown to prevent C5 activation in vivo and in vitro and to protect mice from aPL-induced pregnancy loss (5, 13). To further investigate the role of C5 in thrombophilia induced by aPL, male CD1 mice (weight 25–30 grams; Charles River Laboratories) were injected intraperitoneally with 500 μg of IgG-APS or IgG-NHS (n = 10 mice per group) at time 0 and at 48 hours later. Half of the mice in each group received 1 mg of anti-C5 mAb (BB5.1) and half received 1 mg of murine IgG control at 30 minutes before each injection with IgG-APS or IgG-NHS. Surgical procedures to study thrombus dynamics were performed 72 hours after the first IgG-APS (or IgG-NHS) injection, as previously described (2, 3).

Statistical analysis.

An independent *t*-test was used to compare the antibody levels in different groups of mice. Student's unpaired *t*-test was used to compare the mean thrombus size and number of adhering WBCs between treated and control groups. *P* values of less than 0.05 were considered significant.

RESULTS

Protection of C3- and C5-deficient mice from IgG-APS–induced thrombophilia in vivo.

Consistent with our previous findings, IgG-APS significantly enhanced thrombus size (3.3-fold increase) in $C3^{+/+}$ mice when compared with the effects of treatment with IgG-NHS in $C3^{+/+}$ mice (2, 4). In $C3^{-/-}$ mice treated with IgG-APS, there was a significant reduction ($P = 0.0002$) in the size of injury-induced thrombi (67% decrease) when compared with the effects of IgG-APS in $C3^{+/+}$ mice (Table 1). This reduction in thrombus size in $C3^{-/-}$ mice treated with IgG-APS was similar to that in $C3^{-/-}$ or $C3^{+/+}$ mice treated with IgG-NHS (Table 1).

Table 1. Effects of IgG-APS on thrombus size and leukocyte adhesion to endothelial cells in $C3^{-/-}$ mice*

Mouse type, treatment	No. of animals	Thrombus size, μm^2	WBC adhesion	aCL titer, GPL units†

* Values are the mean ± SD. IgG-APS = IgG from antiphospholipid-positive sera; WBC = white blood cell (expressed as no. of leukocytes adhering within 5 different venules); aCL = anticardiolipin; GPL = IgG phospholipid; WT = wild-type; IgG-NHS = IgG from non-autoimmune healthy sera; C3−/− = deficient in C3.

† Negative titer considered <10 GPL units.

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Mouse type, treatment	No. of animals	Thrombus size, μm²	WBC adhesion	aCL titer, GPL units†
WT				
IgG-NHS	9	802 ± 390	14.0 ± 5.0	<10
IgG-APS	7	2,646 ± 980	35.0 ± 12.0	78.0 ± 8.6
C3$^{-/-}$				
IgG-NHS	7	1,083 ± 443	4.7 ± 0.9	<10
IgG-APS	7	873 ± 425	4.7 ± 2.0	86.3 ± 10.4

* Values are the mean ± SD. IgG-APS = IgG from antiphospholipid-positive sera; WBC = white blood cell (expressed as no. of leukocytes adhering within 5 different venules); aCL = anticardiolipin; GPL = IgG phospholipid; WT = wild-type; IgG-NHS = IgG from non-autoimmune healthy sera; C3−/− = deficient in C3.

† Negative titer considered <10 GPL units.

In C5$^{+/+}$ mice, IgG-APS induced a significant, 2.3-fold increase in thrombus size ($P = 0.003$) when compared with the effects of IgG-NHS in C5$^{+/+}$ mice. The increase in thrombus size induced by IgG-APS was reduced by 54% ($P = 0.011$) in C5$^{-/-}$ mice (Table 2). The thrombus size in C5$^{-/-}$ mice treated with IgG-APS was not different from that in C5$^{-/-}$ or C5$^{+/+}$ mice treated with IgG-NHS (Table 2).

Table 2. Effects of IgG-APS on thrombus size and leukocyte adhesion to endothelial cells in C5$^{-/-}$ mice*

Mouse type, treatment	No. of animals	Thrombus size, μm²	WBC adhesion	aCL titer, GPL units†
WT				
IgG-NHS	7	447 ± 142	1.4 ± 0.4	<10
IgG-APS	10	1,066 ± 141	6.5 ± 2.5	115.7 ± 74.2
C5$^{-/-}$				
IgG-NHS	7	303 ± 248	1.4 ± 0.4	<10
IgG-APS	7	493 ± 149	1.7 ± 0.5	102.3 ± 12.2

* Values are the mean ± SD. See Table 1 for definitions.

† Negative titer considered <10 GPL units.

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Protection of C3- and C5-deficient mice from IgG-APS–induced endothelial cell activation in vivo.

To determine whether complement is required for aPL induction of in vivo endothelial cell activation, we examined WBC adhesion to the endothelium of cremaster muscle. IgG-APS induced an increase in WBC adhesion in both $C3^{+/+}$ and $C5^{+/+}$ mice (2.5-fold [$P = 0.01$] and 4.6-fold [$P = 0.0025$], respectively) when compared with the effects of treatment with IgG-NHS (Tables 1 and 2). In contrast, adhesion of WBCs to cremaster endothelium was significantly reduced after treatment with IgG-APS in C3- and C5-deficient mice (reduction of 87% [$P = 0.002$] and 74% [$P = 0.003$], respectively) (Tables 1 and 2).

The titer of aCL antibodies in $C3^{+/+}$ mice injected with IgG-APS was not different from the aCL titers in $C3^{-/-}$ mice treated with IgG-APS (mean ± SD 78 ± 8.6 IgG phospholipid [GPL] units versus 86.3 ± 10.4 GPL units). Similarly, the levels of aCL antibodies in $C5^{+/+}$ mice treated with IgG-APS were not different from the aCL levels in $C5^{-/-}$ mice treated with IgG-APS (115.7 ± 74.2 GPL units versus 102.3 ± 12.2 GPL units). These findings exclude the possibility that the protective effects of IgG-APS in reducing proinflammatory and thrombogenic events in $C3^{-/-}$ and $C5^{-/-}$ mice were related to lower aCL titers in these mice.

Prevention of IgG-APS–induced thrombosis by anti-C5 mAb.

To confirm that C5 activation is required for the induction of thrombosis by aPL, mice treated with IgG-APS were injected with anti-C5 mAb to inhibit C5 cleavage. In control mice, IgG-APS caused an increase in thrombus size (mean ± SD 3,577 ± 1,129 μm^2 with IgG-APS versus 712.0 ± 272 μm^2 with IgG-NHS; $P = 0.001$) (Figure 1), whereas in mice treated with anti-C5 mAb and IgG-APS, the thrombus size was significantly smaller (838 ± 222 μm^2; $P = 0.005$) when compared with mice treated with control IgG and IgG-APS (Figure 1). The mouse IgG used as the control for the anti-C5 mAb did not affect thrombus size in animals treated with IgG-APS or IgG-NHS. The aCL titer in IgG-APS–treated mice was 152.2 ± 14.8 GPL units and was not different from the aCL titer in mice treated with IgG-APS and anti-C5 mAb (135.3 ± 20.3 GPL units), thus excluding the possibility that the protective effects of anti-C5 mAb were due to a diminution in aCL titers because of interferences between IgG-APS and anti-C5 mAb.

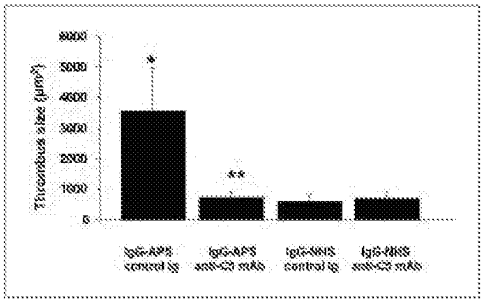

Figure 1.

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Effects of anti-C5 monoclonal antibody (mAb) on thrombosis induced by IgG from patients with antiphospholipid syndrome (IgG-APS). CD1 mice (5 per group) were injected with 1 mg of anti-C5 mAb or 1 mg of murine IgG control. After 30 minutes, the mice were injected with IgG-APS or with IgG from a non-autoimmune healthy individual (IgG-NHS) twice (at time 0 and 48 hours later). The size of induced thrombi (in μm^2) was measured as described in Materials and Methods. Bars show the mean and SEM. $* = P = 0.001$, IgG-APS control Ig versus IgG-NHS control Ig; $** = P = 0.005$, IgG-APS anti-C5 mAb versus IgG-APS control Ig.

DISCUSSION

Using a model of surgically induced thrombus formation, we demonstrated that complement activation plays an important role in thrombosis induced by aPL in mice. Specifically, we identified C3 and C5 as the critical intermediaries linking pathogenic aPL to WBC adhesion and development of thrombosis. Our conclusions are based on the prevention of thrombophilia observed in $C3^{-/-}$ and $C5^{-/-}$ mice and the protective effects of anti-C5 mAb. In previous studies from our group, the findings suggested that C3 activation is required for aPL-induced thrombosis. We demonstrated that Crry-Ig, an inhibitor of C3 convertase, blocks thrombosis initiated by aPL (4). Subsequently, Girardi et al showed that complement activation, specifically C5a–C5aR interaction, is required for aPL-induced pregnancy loss and suggested that C5a promotes neutrophil infiltration of decidual tissue (5). Evidence that neutrophils activated by C5a release procoagulant substances and that monocytes activated by C5a release tissue factor suggests that infiltrating leukocytes stimulated by complement split products can initiate placental infarction and ultimately cause fetal death (8).

In the current study, we have extended our findings by demonstrating that complement is required for aPL-mediated thrombosis and for increased leukocyte adhesion to endothelium. In the absence of C3 or C5, we observed neither enhanced leukocyte adherence nor increased thrombosis associated with aPL treatment. Furthermore, we found that anti-C5 mAb prevented aPL-mediated thrombosis, emphasizing the role of C5 (either C5a, C5aR, or C5b–9) in induction of thrombophilia. C5a binding to endothelial cells results in increased expression of P-selectin and markedly increases neutrophil adhesion (14), and binding of C5b to target surfaces initiates assembly of the MAC that triggers proinflammatory signaling pathways and induces a prothrombotic phenotype in vascular tissue (8). Observations that blockade of C5aR prevents thrombus formation and leukocyte accumulation in a rat model of antibody-mediated thrombotic glomerulonephritis underscore the linkage between complement activation and thrombophilia (15).

Thrombosis in APS is sporadic and may occur in any vein or artery of the body. In this study, we used a mouse model of thrombosis induced by a standardized pinch injury in the femoral vein to define the mediators of thrombophilia associated with aPL (2, 3). Recently, other investigators have demonstrated enhancement of thrombosis by aPL in an experimental model of photochemically induced vascular injury in hamsters (16). Patients with APS often have aPL for prolonged periods of time without clinical manifestations, and thrombosis occurs after a triggering event such as an infection, immobilization, or surgery. Therefore, our experimental model of injury-induced thrombosis,

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although artificial, simulates a "second hit" that triggers thrombotic episodes in susceptible patients and mimics sporadic clotting as observed clinically in APS.

We recognize that there are differences between the human and the murine complement systems. However, the anti-C5 mAb (BB5.1) used in these studies has been shown to effectively block C5 activation in vitro and in vivo in mice and in humans (13, 17, 18). Independent of the initiator of the complement cascade, this mAb prevents C5 activation and thus prevents the generation of the potent proinflammatory factors, C5a and C5b–9. Anti-C5 mAb precipitates the 2 chains of C5 from normal mouse serum and inhibits C5-dependent hemolysis in a functional complement test. It has been shown to prevent aPL-induced pregnancy loss, in which thrombosis plays an important role (5).

Anti-C5 biologic therapy has been extensively investigated in several other animal models of complement-mediated diseases, including collagen-induced arthritis and lupus-like autoimmune disease in (NZB/NZW)F_1 mice (19, 20). Eculizumab (5G1.1), the humanized anti-C5 mAb, is considered a potential treatment for several chronic inflammatory diseases, including rheumatoid arthritis and nephritis, and phase II trials have been initiated for these indications. Furthermore, eculizumab has been shown to prevent C5 activation in humans and to have beneficial effects in patients with paroxysmal nocturnal hemoglobinuria; specifically, it reduces intravascular hemolysis, hemoglobinuria, and the need for transfusion in these patients, providing a proof-of-concept that blockade of complement activation is feasible and tolerable in patients with chronic disease (18).

We propose that pathogenic aPL, in addition to their direct effects on platelet and endothelial cell targets, induce complement activation, and thus generate complement split products that attract inflammatory cells and initiate thrombosis and tissue injury. Our finding that blockade of C5 is effective in preventing thrombosis in a mouse model of APS has important therapeutic implications. Blockade of complement activation may be a valuable target for interventions that prevent, arrest, or modify the thrombogenic effects of aPL.

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eculizumab Alexion EMEA grants request for accelerated assessment procedure

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REVIEW

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Complement-targeted therapeutics

Daniel Ricklin & John D Lambris

The complement system is a central component of innate immunity and bridges the innate to the adaptive immune response. However, it can also turn its destructive capabilities against host cells and is involved in numerous diseases and pathological conditions. Modulation of the complement system has been recognized as a promising strategy in drug discovery, and a large number of therapeutic modalities have been developed. However, successful marketing of complement-targeted drugs has proved to be more difficult than initially expected, and many strategies have been discontinued. The US Food and Drug Administration's approval of the first complement-specific drug, an antibody against complement component C5 (eculizumab; Soliris), in March 2007, was a long-awaited breakthrough in the field. Approval of eculizumab validates the complement system as therapeutic target and might facilitate clinical development of other promising drug candidates.

When the bactericidal properties of certain heat-labile blood components were initially described more than a century ago[1], no one could have imagined the considerable impact the complement system would have on both immunology and our understanding of many disease processes. Even the name "complement," originally coined by Paul Ehrlich[2], implied only a supplementary role of this system in the defense against microbial intruders. However, continued progress in research has led to a dramatic change in our knowledge about the complement system and moved it into the spotlight of basic and applied life sciences.

Today it is clear that complement is a key player of the innate immune system. However, this integral position in the maintenance and regulation of immune and inflammation reactions also makes it a trigger point for a variety of pathologic conditions. Erroneous activation or insufficient regulation of the complement cascade may turn its destructive actions against the host's cells. As a consequence, many inflammatory and autoimmune diseases are thought to be caused, or at least supported, by unleashed complement. Inhibition or modulation of complement activity has therefore been recognized as a promising therapeutic strategy for many years.

Although many creative attempts to design complement-specific drugs have been made in recent decades, their development from experimental concept to clinical product has faced many obstacles. In light of these complications, most of the major pharmaceutical companies seem to have abandoned their initial efforts to develop drugs that target complement. Despite its bitter taste, this decision has encouraged a series of small startup companies in the biotechnology field to take up the challenge. The first results of this commitment are now visible and may well lead to a revival of this difficult but promising area of drug discovery. With its 2007 approval of an antibody against complement component C5 (eculizumab; Alexion, Cheshire, CT, USA), the US Food and Drug Administration (FDA) has now authorized the first complement-specific drug[3]. Many other promising drug candidates and therapeutic strategies are currently in the pipeline of various companies. The aim of this review is to provide an overview of current therapeutic strategies, with a focus on drug candidates that are in clinical trials (**Table 1**) or late preclinical development (**Table 2**).

Department of Pathology and Laboratory Medicine, School of Medicine, University of Pennsylvania, 401 Stellar Chance, 422 Curie Blvd., Philadelphia, Pennsylvania 19104, USA. Correspondence should be addressed to J.D.L. (lambris@mail.med.upenn.edu).

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The complement system in innate and adaptive immunity

With an estimated age of 600–700 million years, the complement system is one of the most ancient defense strategies[4], yet it represents far more than merely a simple protection mechanism against microbial infections. As an essential connection between adaptive and innate immunity, complement components are able to orchestrate immune reactions by communicating with multiple immune cells (**Fig. 1**). In addition, complement contributes to the maintenance of homeostasis by recognizing and eliminating apoptotic and necrotic cells. Furthermore, through its activity, the solubility of circulating immune complexes is maintained, thereby facilitating their elimination[5–9].

As a first line of defense against pathogens, complement has to quickly detect, tag, destroy and eliminate microbes. This task can be accomplished only by a complex and tightly regulated assembly of proteins involving various soluble and surface-bound complement components, receptors and regulators (**Fig. 2**). Complement activation is usually initiated by the interaction of several pattern-recognition receptors with foreign surface structures. Depending on the activation trigger, the complement cascade follows one of three pathways: the classical, lectin or alternative pathway.

The recognition of antibody complexes on the surface of pathogens, mediated by the multimeric collectin C1q, was early recognized as initiator of an enzymatic cascade that was later termed classical pathway. In the related lectin pathway, complement activation is initiated by an interaction of mannose binding lectin (MBL) with high-density arrays of terminal mannose on bacterial surfaces. In both cases, these pattern-recognition proteins form complexes with serine proteases (C1r/C1s and MASP, respectively) that lead to the cleavage of C4 to its fragments C4b and C4a. After binding of C2 to C4b, the same protease complexes are responsible for generating the active C2a fragment, which is part of the classical pathway C3 convertase (C4bC2a; **Fig. 2a**). To guarantee instant response to pathogens, the cascade is constantly kept at a low level of

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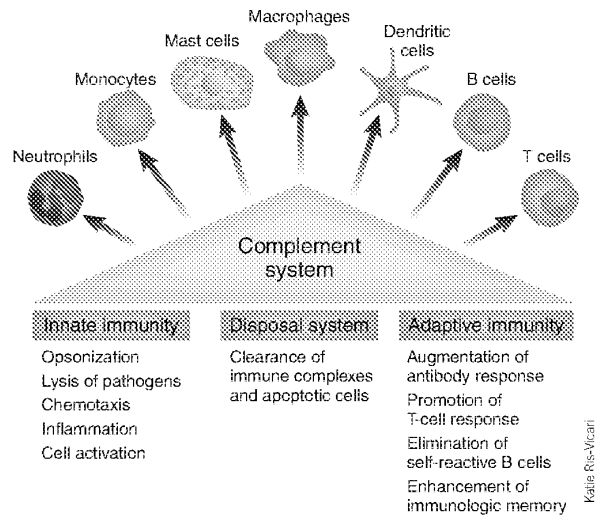

Figure 1 The complement system as a bridge between innate and adaptive immunity. Although the complement system has traditionally been considered part of the innate immune system, research in recent decades has revealed that complement is able to activate cells involved in both the adaptive and innate immune response. Complement triggers and modulates a variety of immune activities and acts as a linker between the two branches of the immune response. In addition, the complement system maintains cell homeostasis by eliminating cellular debris and immune complexes.

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activity—'tick-over'—by the alternative pathway. Spontaneous hydrolysis of C3 to C3w induces a conformational change that enables binding to factor B and formation of the initial alternative pathway C3 convertase (C3wBb; **Fig. 2a**). The alternative pathway may represent up to 80% of complement activation in some instances and can also be triggered by various reactions with foreign surfaces.

All three pathways converge in the cleavage of C3 into its active fragments, C3a and C3b, by the C3 convertases. Formation of C3b exposes a previously hidden thioester group that covalently binds to patches of hydroxyl and amino groups on the pathogen surface. This process, termed opsonization, is critical for all subsequent steps in the complement cascade and the elimination of pathogens and apoptotic host cells by phagocytes. In an amplification loop, binding of factor B to surface-bound C3b and its cleavage by factor D generates the alternative pathway C3 convertase C3bBb, which causes a gradual acceleration of C3b production. Surface-tethered C3b also plays a central role in the formation of the two C5 convertases (C4bC2aC3b and C3bC3bBb), which cleave C5 into C5a and C5b. C5b initiates the assembly of the membrane attack complex (MAC), a pore that is formed by components C5b, C6, C7, C8 and multiple units of C9 and ultimately leads to cell lysis. The anaphylatoxins C3a and C5a, generated as a result of proteolytic cleavage of C3 and C5, respectively, are potent inflammatory mediators that trigger a plethora of processes, which culminate in the stimulation of immune cells and the elimination of the pathogen[5].

Despite years of intensive research in this field, our picture of complement activation seems to be far from complete. Very recently, additional activation pathways have been identified: for example, MBL-associated protease 2 (MASP-2) of the lectin pathway seems to directly attack and cleave C3 without formation of the corresponding C3 convertase (C2 bypass pathway; **Fig. 2a**)[10]. Similarly, direct cleavage of C3 and C5 by a series of extrinsic proteases such as kallikrein or thrombin (extrinsic protease pathway; **Fig. 2a**), has also been proposed. This activity points to an interesting connection to other essential plasma cascades such as the coagulation system[11]. Finally, properdin seems to not only stabilize the C3 convertase as a positive complement regulator but may also act as a pattern-recognition molecule and actively induce convertase formation on foreign surfaces[12,13].

One of the most fascinating aspects of complement is the sophisticated regulation mechanism that allows a rapid reaction to pathogenic intruders while protecting host cells from its destructive potential. After activation, the opsonization rates often reach several thousand C3b molecules per cell per minute. In principle, this attack is not specifically directed against foreign cells. However, interplay of time, location and molecule-based regulation mechanisms generally prevents the complement cascade from attacking host tissues. Both the thioester group of C3b and the C3 convertases have short half-lives, limiting their action to the site of activation. Whereas the convertase is actively stabilized on foreign cells by properdin (**Fig. 2a**), a set of soluble and membrane-bound negative regulators inactivates and degrades active molecules on host cells (**Fig. 2b**). These structurally related regulators of complement activation include factor H, decay accelerating factor (DAF/CD55), membrane cofactor protein (MCP/CD46), C4-binding protein (C4BP) and complement receptor 1 (CR1/CD35). These regulators either induce an accelerated decay of the convertases (DAF, CR1, factor H) or act as cofactors for factor I, which degrades C3b to its inactive form iC3b (MCP, CR1, factor H). An additional regulator of complement activation, CD59, prevents the formation of MAC by intercalating between C8 and C9 subunits (**Fig. 2b**)[5,14,15].

Complement's involvement in disease

The central role of complement in the orchestration of immune reactions inevitably renders it a target for immune evasion or attack by pathogens and also a contributor to many diseases. Even small disruptions in the delicate balance of activation and regulation can lead to an overreaction of complement that triggers inflammation and cell lysis. Many inflammatory, autoimmune, neurodegenerative and infectious diseases have been shown to be associated with excessive complement activity (**Fig. 3**). Complement involvement is usually complex and may include both an inappropriate initiation of the cascade and deficiencies in specific components or regulators[6,16,17].

Ischemia/reperfusion (I/R) injury is a severe consequence of conditions that lead to a deprivation and subsequent restoration of the tissues' blood supply; I/R injury can occur in a number of clinical conditions, including acute myocardial infarction, stroke, and hemorrhagic and septic shock, and may also be a complication of coronary artery bypass graft surgery. Paradoxically, it appears that the tissue reperfusion and not the ischemia activates complement and leads to inflammation-induced damage. Even though the exact involvement of complement activation in I/R injury is still unclear, several experimental studies have indicated a connection between complement and the pathogenesis of I/R injury, and have suggested complement inhibition as a potential therapy[18].

Complement seems to be a major contributor to a number of autoimmune diseases. For example, deposition of immune complexes during systemic lupus erythematosus initiates the classical pathway by binding of C1q (**Fig. 2b**) to these complexes[19]. A similar mechanism may also be involved in the pathogenesis of rheumatoid arthritis, and C5a (**Fig. 2b**) seems to have a major role in psoriasis and complement-mediated inflammatory diseases including asthma[20]. Complement activation by C1q, which acts as a pattern-recognition receptor for amyloidal plaques, has been correlated with the pathology of Alzheimer's disease[21]. Although complement also seems to be involved in other neurodegenerative diseases such as Huntington's disease or Parkinson's, the exact connection is less clear[21].

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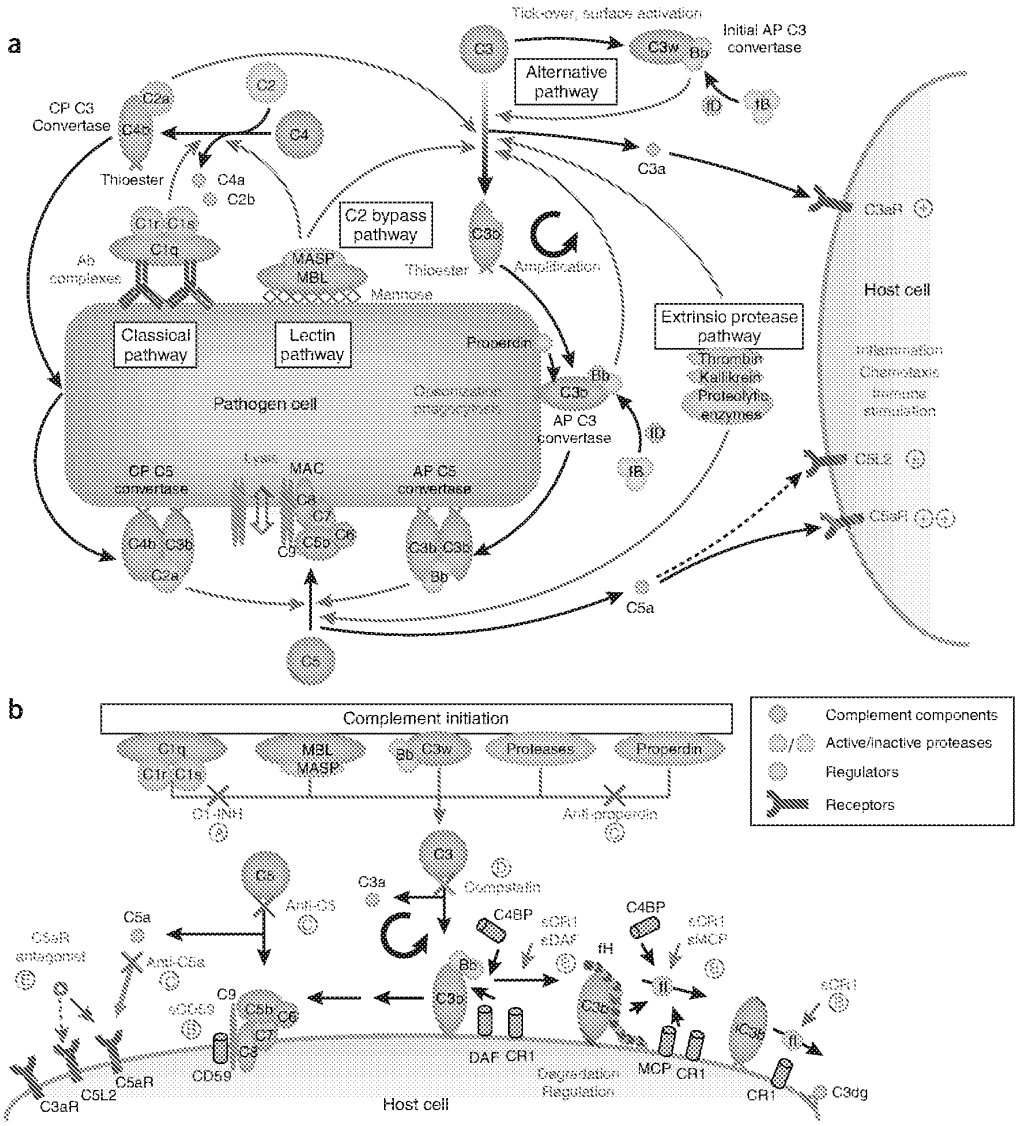

Figure 2 Activation, regulation and therapeutic modulation of the complement system. (**a**) The complement cascade after activation by pathogens. In addition to a low level of constant activation by the alternative pathway (AP; tick-over via hydrolyzed C3 (C3w) results in the formation of the initial C3 convertase C3wBb), the complement cascade is usually activated by antibody complexes (classical pathway) or high-density mannose (lectin pathway) on the surface of pathogens. This activation leads to the formation of the C3 convertases C4bC2a and C3wBb, which cleave native C3 to C3b and C3a. Deposition of C3b on cell surfaces via its thioester group initiates the cleavage of more C3 (the amplification loop via the final AP C3 convertase C3bBb), opsonization and phagocytosis, as well as lysis as a result of the formation of the membrane attack complex (MAC). In addition, the anaphylatoxins C3a and C5a are released and trigger further immune reactions upon binding to their receptors (C3aR, C5aR, C5L2). These combined actions of complement lead to the elimination of pathogenic cells. Recently, studies have shown that some steps of the cascade can be directly initiated by certain proteolytic enzymes (the extrinsic protease pathway) or by MBL/MASP (the C2 bypass pathway). Furthermore, the regulatory protein properdin may propagate and stabilize the formation of C3 convertases on the surface of the cell. Protein conversions are shown as black arrows and enzymatic reactions as green arrows. (**b**) Regulation, deactivation and inhibition of the complement cascade on host cells by natural regulators and complement-specific therapeutics. Several pathogenic processes and diseases are the result of an erroneous activation or insufficient downregulation of the complement cascade. Under normal conditions, any host-associated C3 convertase (C3bBb) undergoes an accelerated decay mediated by complement receptor 1 (CR1), decay accelerating factor (DAF), C4b-binding protein (C4BP) or factor H (fH). C3b is degraded to inactive iC3b by factor I in a reaction that requires as cofactor CR1, fH, C4BP or membrane cofactor protein (MCP). In addition, CD59 prevents the formation of the MAC. Some of the therapeutic interventions focus on increasing this downregulation by using soluble forms of these regulators (that is, sCR1, sDAF, sMCP, sCD59). Other approaches involve the substitution of the natural C1 inhibitor (C1-INH), the inhibition of the central conversion of C3 to C3b and C3a (compstatin), blockage of C5 or C5a by antibodies, and the suppression of anaphylatoxic signaling by C5a receptor antagonists. For clarity, only the regulation of the alternative pathway, which may contribute up to ~80% of all complement activity, is shown here. The C3 convertase of the classical pathway (C4bC2a), as well as the C5 convertases (C4bC2aC3b and C3bC3bBb), can be modulated by the same regulators and drug compounds. Therapeutic modulators in clinical trials or late preclinical development are indicated in red, with the circled letter referring to the corresponding drug class in **Tables 1** and **2** and the main text.

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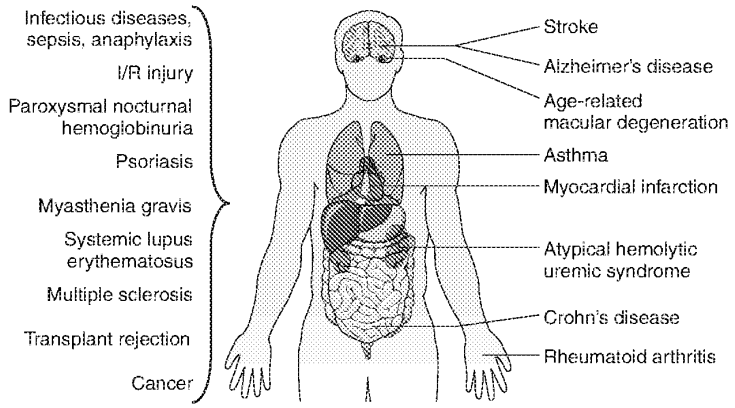

Figure 3 Examples of pathological conditions involving the complement system. Erroneous activation or insufficient regulation of the complement cascade may lead to an attack by the immune system against self-tissue. Many autoimmune, inflammatory and ischemia/reperfusion (I/R) injury-related diseases are therefore connected with complement. Whereas some of these pathological conditions are localized to specific organs and tissues, many of them are systemic. In addition, some pathogens have found ways to evade or even misuse the complement system, thereby contributing to infectious diseases and their consequences.

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Alterations in the expression and functions of complement regulators may be another cause of complement-related diseases. For example, polymorphism and mutations in genes encoding factor H (**Fig. 2b**) are associated with age-related macular degeneration (AMD), the major cause of blindness in industrial nations, and atypical hemolytic uremic syndrome (aHUS), respectively[22]. Low plasma levels of C1 inhibitor have been found to be responsible for hereditary angioedema (HAE)[23,24], and a lack of CD59 (**Fig. 2b**) on erythrocyte surfaces causes paroxysmal nocturnal hemoglobinuria (PNH)[25].

Finally, a series of pathogens have found ways to evade or even hijack complement proteins. Microbial proteins that either inhibit complement components or mimic regulators have been identified in both viruses (e.g., HIV, smallpox, Epstein-Barr virus) and bacteria (e.g., *Staphylococcus aureus, Streptococcus pneumoniae*)[5,26–30].

Druggability of complement

The central role of complement in the pathophysiology of major diseases makes it an interesting target for the pharmaceutical industry. However, despite numerous attempts in recent years to inhibit or modulate complement therapeutically[17,31–34], the success rate has been disappointingly low. Before the recent approval of complement-specific drugs, many promising candidates encountered insuperable obstacles while in clinical development. Inevitably, this high attrition rate raises questions about what renders complement drug discovery such a difficult task.

The multifaceted nature of both the cascade and its disease involvement may be one central problem. Many questions about the exact disease-related mechanisms of complement, both at the molecular and clinical level, are still unresolved, and this lack of clarity complicates specific targeting. "Druggability" (**Box 1**) is another major issue. Traditional drug discovery aims for small molecular entities with the potential for oral administration, yet many critical steps in the complement cascade are fundamentally based on large protein-protein interactions, which are challenging to influence with small molecules[35]. Anaphylatoxin receptors and serine proteases are the only complement targets with an auspicious druggability potential. This limitation may also explain the exceptionally high number of biopharmaceuticals (proteins, antibodies, peptides, nucleotides) and biotechnology startups that are involved in the current drug development process (**Tables 1** and **2**). Finally, the optimal target within the complement cascade (**Fig. 2b**) and the extent of inhibition has to be critically defined for each indication. Suppressing pathogenic complement activity without compromising its defensive and immunomodulatory functions might be one of the most delicate challenges when treating chronic conditions. Intervention at the activation level may require a highly specific approach, for example, inhibiting the recognition of amyloid plaques by C1q in the case of Alzheimer's disease. However, inhibition of a single pathway may be insufficient for many diseases and can still put pathogen defense at risk. Inhibition of C3 and its convertase potentially has the broadest effect, because it blocks all terminal activity of complement as well as the amplification loop. However, this nearly complete blockage may also lead to adverse effects and therefore favors local or targeted applications. Prevention of terminal complement action, for example, by inhibiting C5a formation and binding, is highly specific but requires the most information about the target involved. Finally, supplementation or inhibition of complement regulators may offer a more physiologic and specific way to modulate complement activity, especially in the treatment of chronic diseases.

Current complement therapeutics

A number of promising approaches for the clinical substitution, inhibition or modulation of complement have been developed and are presented in the following sections. We have classified complement therapeutics that either have been in clinical trials (**Table 1**) or are in the late steps of preclinical development (**Table 2**) into five major categories according to the mechanism of action (labeled **a–e** in **Tables 1** and **2**, and **Fig. 2b**).

Serine protease inhibitors. The consecutive cleavage and activation of several proteases constitutes the driving force behind complement function. Eight serine proteases are integral elements of the complement cascade itself (C1r, C1s, C2a, MASP-1, MASP-2, factor D, factor B, factor I)[36], whereas others are involved through the extrinsic protease pathway (**Fig. 2**)[11]. Because proteases are among the most druggable targets in the complement system (**Box 1**), it is not surprising that early drug development efforts focused on protease inhibitors[37]. Although screening- and structure-based approaches resulted in drug candidates for factor D (BCX-1470, BioCryst, Birmingham, AL, USA)[38] and C1s (C1s-INH-248, Knoll/Abbott, Abbott Park, IL, USA)[39], short half-lives and a lack of specificity have thus far prevented the successful development of small-molecule protease inhibitors for the treatment of complement-related disorders.

The only complement-associated protease inhibitor currently on the market is C1-inhibitor (C1-INH), a heavily glycosylated plasma protein that is used in the treatment of HAE[23,24,40,41]. This disease is characterized by recurrent episodes of severe skin and mucous membrane edema and is caused by either an insufficient production of C1-INH (type 1) or a mutation around its binding site that leads to inactivity (type 2). In both types, therapeutic supplementation of this protein has proven to be an effective and safe treatment for HAE and helps prevent severe disease

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relapses and life-threatening complications[42,43]. A third HAE type of unknown etiology and low prevalence does not benefit from supplementation, because it is not dependent on C1-INH activity[44]. Although C1-INH gained its name from its ability to block the serine esterase activities of C1r and C1s[45], its specificity includes other proteases, such as MASP, kallikrein and coagulation factors XI and XII. Although plasma levels of C2 and C4 are clearly affected by HAE, the primary mechanism of C1-INH seems to be more closely associated with bradykinin-kallikrein than with the complement system[23]. However, there is increasing evidence that therapeutic administration of C1-INH may also be beneficial in other disorders with a direct connection to complement, such as I/R injury[41,46–48].

Whereas pasteurized C1-INH concentrates for the treatment of HAE have been available since 1985, a lack of licensed products still limits the accessibility of this treatment in several countries, including the United States[43]. Fortunately, annotation of orphan drug status by the FDA (**Box 2**) and licensing agreements with established providers have boosted the development and registration of this important drug, and its approval in the United States is imminent[49]. Current C1-INH formulations are still being improved[50], for example, by the use of nanofiltered concentrates that feature better safety with regard to viral contamination. The high production costs and extensive quality control requirements for plasma-derived C1-INH still makes it an expensive treatment option and will certainly limit its availability in poorer countries. Development of a recombinant form, which is isolated as a glycosylated protein from the milk of transgenic animals, may be an important step in overcoming these limitations[51]. Experience with C1-INH in the clinic and its good safety profile are expected to lead to an extension of its indications. For example, clinical trials regarding the use of C1-INH for the prevention of I/R injury after acute myocardial infarction are currently planned.

Table 1 Complement therapeutics on the market or in clinical trials

Product (company)	Activity	Stage of development
Protease inhibitors (A)		
C1-INH (Cetor/Sanquin, BerinertP/CSL Behring, Lev Pharma)	Purified plasma protein; inhibition of C1r/C1s, kallikrein and other proteases	Marketed (HAE)[a]; clinical phase 3 (HAE)[a]; preclinical/phase 1 for other indications (AMI, CABG)
Rhucin/rhC1INH (Pharming Group N.V.)	Recombinant human C1-INH from transgenic rabbits	Clinical phase 3 (HAE)
Soluble complement regulators (B)		
sCR1/TP10 (Avant Immunotherapeutics)	Extracellular part of CR1; decay accelerator, factor I cofactor	Clinical phase 2 (CABG)[b]
CAB-2/MLN-2222 (Millenium Pharmaceuticals)	Chimera of DAF and MCP; decay accelerator, factor I cofactor	Clinical phase 1 (CABG)[b]
Therapeutic antibodies (C)		
Eculizumab/ Soliris (Alexion Pharmaceuticals)	Humanized long-acting mAb against C5	Marketed (PNH), preclinical for other indications
Pexelizumab (Alexion Pharmaceuticals)	Humanized short-acting mAb against C5	Clinical phase 3 (AMI, CABG)[b]
Ofatumumab (Genmab A/S)	Humanized anti-CD20 mAb	Clinical phase 2
Complement component inhibitors (D)		
Compstatin/POT-4 (Potentia Pharmaceuticals)	Peptidic C3 inhibitor; no generation of C3a/C3b	Clinical phase 1 (AMD)
Receptor antagonists (E)		
PMX-53 (Peptech Ltd.)	Peptidic C5aR antagonist	Clinical phase 2 (RA, psoriasis)
Other		
rhMBL (Enzon Pharmaceuticals)	Recombinant human MBL as substitution therapy	Clinical phase 1b (MBL deficiency)

[a]C1-INH preparation are only approved in some countries, whereas they are in clinical phase 3 in the USA. [b]Clinical trials have recently been performed but no product development has been undertaken since then, or the development was officially discontinued. AMD, age-related macular degeneration; AMI, acute myocardial infarction; CABG, coronary artery bypass grafting; HAE, hereditary angioedema; PNH, paroxysmal nocturnal hemoglobinuria; RA, rheumatoid arthritis.

Soluble complement regulators. Because regulators of complement activation are natural modifiers of complement activities and prevent a host cell from being attacked by its own defense system, they have been considered for therapeutic use since the early stages of complement drug discovery[52]. A first breakthrough was reached with the expression of a soluble form of complement receptor 1 (sCR1)[53]. This molecule featured both decay accelerator and cofactor activity and had a high potency in inhibiting both the classical and alternative pathways. sCR1 showed promising results in the treatment of I/R injury and various other conditions in experimental animal models. Based on these encouraging results, sCR1 was developed as a therapeutic (TP10; Avant Immunotherapeutics, Needham, MA, USA) for use after coronary artery bypass graft surgery. TP10 is expressed as a 240-kDa glycoprotein in Chinese hamster ovary cells, has a plasma half-life of ~55 hours and is safe and well-tolerated in both adult and infant patients[54]. In a large placebo-controlled phase 2 trial, comprising 564 high-risk patients undergoing cardiac surgery, a single intravenous bolus of TP10 immediately before surgery was found to inhibit complement activation for up to 3 days postoperatively. Whereas TP10 treatment resulted in a significant improvement of the clinical endpoints in males, no such benefits were observed in female participants. This surprising gender specificity was confirmed by a subsequent phase 2b study comprising 297 women. Whereas the mechanism governing these differences is still unclear, effects other than complement inhibition are likely to be involved. As a consequence, a male-only indication of TP10 for bypass surgery was considered for clinical phase 3. TP10 also showed promising results in smaller studies of adults undergoing lung transplants and infants undergoing coronary artery bypass graft surgery[54]. However, Avant Immune has recently decided not to continue investing in clinical trials of TP10 (ref. 55). It remains to be seen whether this development will mean the end of sCR1 as a therapeutic compound. Improvements in the structure (e.g., a reduction of its size) and formulation of sCR1 or an extension of its indications may well lead to its resurrection. Although the first steps in this direction have already been taken, neither TP20 (sCR1 with sialyl Lewis^X-glycosylation for targeting sites of inflammation[56], Avant Immune) nor Mirococept/APT070 (a truncated sCR1 with a membrane-tethering motif[57], Inflazyme Pharmaceuticals, Richmond, BC, Canada) has been tested in clinical trials.

Soluble forms of MCP, DAF and CD59 have also been considered as therapeutics. Whereas DAF and MCP each offer only a single regulatory activity, a recombinant chimera of their extracellular parts has been developed[17]. The resulting sDAF-sMCP hybrid was initially named "complement activity blocker 2" (CAB-2; Xoma, Berkeley, CA, USA) but entered clinical trials with a new name and licensing partner (MLN-2222; Millennium, Cambridge, MA, USA). However, no further studies have been initiated recently. Like Mirococept/APT070, a membrane-tethering sCD59 has been developed by Inflazyme Pharmaceuticals. Although

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Table 2 Complement therapeutics in pre-clinical development

Product (company)	Activity	Stage of development
Protease inhibitors (A)		
Factor D inhibitors[a] (BCX1470 etc.)	Small molecule serine protease inhibitors	Mostly discontinued (due to lack of specificity and/or short half-life)
Soluble complement regulators (B)		
sCR1-sLe^x/TP-20 (Avant Immunotherapeutics)	sCR1 with sLe^x-rich glycosylation to target sites of inflammation	Development/preclinical (stroke, heart attack)
Mirococept (Inflazyme Pharmaceuticals)	sCR1 with lipopeptide membrane linker	Preclinical (AMI, inflammatory diseases)
Therapeutic antibodies (C)		
TNX-234 (Tanox)	Humanized antibody against factor D	Preclinical (wet AMD)
TNX-558 (Tanox)	Humanized antibody against C5a	Development/preclinical (inflammatory diseases)
TA106 (Taligen Therapeutics)	Antibody against factor B	Development/preclinical
Neutrazumab (G2 Therapies)	Antibody blocking the C5a receptor	Development/preclinical (RA, stroke)
Anti-properdin (Novelmed Therapeutics)	Antibody against properdin	Development/preclinical
HuMax-CD38 (Genmab A/S)	Humanized anti-CD38 mAb that triggers complement	Preclinical studies (cancer, multiple myeloma)
Complement component inhibitors (D)		
ARC1905 (Archemix)	Aptamer-based C5 inhibitor (PEG-ylated)	Preclinical (AMD)
Receptor antagonists (E)		
JPE-1375, JSM-7717 (Jerini[b])	Small molecule/peptidomimetic antagonists for C5a receptor	Preclinical (inflammation, renal and ocular diseases[b])

[a]Currently, there are no complement-specific serine protease inhibitors listed in the pipelines of pharmaceutical companies. [b]Ophthalmology compounds are developed further by Jerini Ophthalmic.

not listed in the company's pipeline, experimental studies were recently performed to investigate its potential therapeutic usefulness as a treatment option for PNH[58]. This rare, genetic, life-threatening blood disorder leads to decreased expression of membrane-anchored proteins, including CD59 and DAF, on erythrocytes. Substitution and membrane tethering of recombinant CD59 may develop into a promising therapy for PNH[58].

Therapeutic antibodies. Following a general trend in the pharmaceutical industry, antibody-based therapeutics appear to be the most rapidly growing drug class against complement-related diseases. Crucial developments in their screening, production and humanization have led to a remarkable boost in the number of therapeutic antibodies[59]. By targeting specific components of the complement system, all stages from the initiation and activation process to single terminal actions can hypothetically be blocked in a selective manner. Current drug candidates in the pipeline focus primarily on the inhibition of downstream processes around C5 and its cleavage fragment, the anaphylatoxin C5a.

Selective inhibition of C5 using monoclonal antibodies (mAb) has been considered a promising therapeutic option for many years. A highly selective mAb against mouse C5 was introduced by Frei *et al.* two decades ago[60] and was later demonstrated to be effective in a mouse model of rheumatoid arthritis by Alexion Pharmaceuticals[61]. Continuous improvement and clinical testing finally led to the FDA approval of eculizumab[3], which is currently the only complement-specific antibody on the market[62]. Eculizumab is the first and only approved therapy for PNH, a rare but life-threatening disorder that is characterized by a chronic destruction of red blood cells. A mutation on the X chromosomes of hematopoietic stem cells prevents the proper biosynthesis of the glycosylphosphatidylinositol (GPI) anchor, which leads to a deficiency in membrane-anchored proteins, including DAF and CD59. The lack of efficient inhibition of complement activation on red blood cells results in an increase in MAC formation on erythrocyte membranes and cell lysis. The survival of PNH erythrocytes is dramatically reduced in PNH patients, to 10% of normal red blood cells. On platelets, the absence of CD59 and increased MAC formation can lead to morphological changes of their surface. These platelets show enhanced susceptibility for activation, contributing to a high risk of thrombosis in PNH patients. Until very recently, only supportive therapies (blood transfusions, iron therapy, anticoagulation and others) or allogeneic stem cell transplantation had been available for the clinical management of this disease[25].

Given the complement-focused molecular mechanism of PNH, prevention of MAC activity evolved as the most promising treatment strategy. Two possible approaches were identified: substitution of defective CD59 or neutralization of complement proteins involved in MAC formation itself, as in the case of eculizumab. By tightly binding to C5 and preventing its cleavage to C5b, this new drug suppresses the formation of the MAC and reduces the clinical symptoms of PNH. Eculizumab (5G1.1-SC) is a humanized version of the anti-C5 antibody h5G1.1, which was first described in 1996 (ref. 63). Eculizumab received orphan drug status for PNH from both the FDA and the EU in 2003, and Alexion Pharmaceuticals subsequently initiated two phase 3 trials. In both studies, eculizumab was safe and well-tolerated, and no development of antibodies against the drug was detected. Because complement inhibition is expected to lead to a higher susceptibility to infection, particularly with *Neisseria meningitidis*, all patients had been vaccinated with a meningococcal vaccine before beginning treatment. The antibody was clearly effective: 51% of those who received eculizumab gained independence from blood transfusions (versus 0% in the placebo group). Both the endogenous erythrocyte mass and the hemoglobin levels were remarkably higher during treatment than before, and many symptoms related to quality of life (e.g., fatigue, abdominal pain) were attenuated. Whereas the preliminary data also indicated a lower risk for thrombosis, this benefit needs to be confirmed by ongoing clinical trials[25,64,65].

As an anti-C5 antibody that inhibits the generation of both C5b and the anaphylatoxin C5a, the potential indications for eculizumab are certainly not limited to PNH. Consequently, eculizumab has undergone several preclinical and clinical studies for a variety of conditions (e.g., psoriasis, rheumatoid arthritis, systemic lupus erythematosus and transplant rejection). Even though some of these paths seem to have been discontinued[65], Alexion Pharmaceuticals still performs preclinical development of eculizumab for autoimmune diseases and transplant rejection. Furthermore, nebulized eculizumab for the treatment of asthma and an intravitreal application to treat age-related macular degeneration are in the company's pipeline. In the case of pexelizumab, the short-acting sc-Fv fragment of eculizumab, several phase 3 trials have been done to test its potential for use in coronary artery bypass graft surgery and acute

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myocardial infarction[66]. Despite promising preclinical results, however, data from these clinical trials did not show significant improvement in the treatment group when compared to the placebo group[67,68]. Alexion Pharmaceuticals has therefore announced that it plans to focus its development efforts on eculizumab[69].

The concept of using humanized antibodies (and their fragments) to inhibit certain steps of the complement cascade seems to be a very productive strategy. Several companies list anticomplement antibodies in their pipelines (**Table 2**). Two of them target the various actions of the anaphylatoxin C5a. Neutrazumab (G2 Therapies, Darlinghurst, NSW, Australia) binds to extracellular loops of C5aR and thereby inhibits the binding of C5a to its major signaling receptor. G2 Therapies recently announced a partnership with Novo Nordisk (Bagsværd, Denmark) with regard to the development of their anti-C5aR antibodies. Another approach has been taken by Tanox (now part of Genentech, S. San Francisco, CA, USA) with their antibody TNX-558, which neutralizes C5a by binding to the anaphylatoxin itself. Interestingly, TNX-558 binds to a C5a epitope in native C5 but does not prevent its cleavage. Tanox has also developed an antibody directed against factor D (TNX-234). Together with the anti–factor B (TA106 for age-related macular degeneration and asthma[70]; Taligen, Aurora, CO, USA) and anti-properdin (Novelmed, Cleveland, OH, USA) antibodies in development, these biologics may offer additional approaches for inhibiting complement at the C3 convertase level.

Whereas inhibition of complement activity is the desired outcome in the vast majority of therapeutic approaches, its local stimulation may be beneficial in some malignant diseases, such as cancer. Experience with monoclonal antibodies in cancer therapy suggests that induction of cell death through antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity (CDC) may be a major driving force behind their effectiveness (e.g., in the case of the anti-CD20 mAb rituximab from Roche). However, relatively high antigen densities are required for an efficient initiation of the classical pathway by C1q. Genmab (Copenhagen) recently described anti-CD20 antibodies with exceptionally high CDC potency[71,72]. Both the location of the epitope as well as the dissociation rate are apparently responsible for this high level of effectiveness. Ofatumumab/HuMax-CD20 has been selected for clinical development and has recently been evaluated in terms of its effectiveness in treating acute rheumatoid arthritis (phase 2), B-cell chronic lymphocytic leukemia (phase 3) and follicular lymphoma (phase 3). Other antibodies with high CDC potency against multiple myeloma (HuMax-CD38) or colon, pancreatic and prostate cancer (HuMax-ZP3) are currently in preclinical development (http://www.genmab.com/). This strategy is a good example of how to make use of a more general, nonspecific property and select and optimize it to obtain the desired clinical effect. Because CDC may generally be induced by therapeutic IgGs (and IgMs), the CDC activity of these reagents may become a crucial parameter in the development of therapeutic antibodies.

Complement component inhibitors. Although antibodies may be considered the archetype of site-blocking compounds, smaller molecules such as peptides, nucleotides and synthetic molecules may also have the potential to interrupt protein functions by steric hindrance or the induction of conformational changes. Small functional inhibitors of complement activity are expected to have drug-like properties with enhanced pharmacokinetic profiles (**Box 1**). These advantages may make them suitable for drug development efforts related to oral bioavailability and better administration routes.

Compstatin is the most developed candidate in this class of substances and recently entered clinical trials. This cyclic tridecapeptide has been discovered through screening of combinatorial phage-display libraries[73] in the research group led by one of the authors of this review. It effectively prevents the cleavage of C3 to its active fragments C3a and C3b and therefore inhibits the most central step in the complement cascade. Although the exact mechanism of compstatin's inhibitory action has not yet been resolved, the recent publication of the cocrystal structure between compstatin and C3c suggests that a disruption of protein-protein interactions during convertase formation may be an important determinant[74]. This finding has led to the hypothesis that a conformational change or an interruption in protein-protein interactions is responsible for its activity. Compstatin has shown effective complement inhibition in a variety of experimental disease models[34]. Despite a narrow selectivity for primate C3 that may affect preclinical animal experiments[75], its high efficacy and rather small size make it a promising drug candidate. Its inhibitory efficacy has been increased by 264-fold[34,76] through the use of rational and combinatorial synthesis, structural analysis, and computational approaches to identify active analogs. Potentia (Louisville, KY, USA) has announced the inauguration of clinical trials for the treatment of neovascular age-related macular degeneration by intravitreal application of a compstatin analog (POT-4).

Combinatorial approaches have also been used to identify a short aptamer molecule directed against C5 (ref. 78). Aptamers are single-stranded nucleotide stretches that have molecular recognition properties similar to those of antibodies, but offer better options for derivatization. They can be selected in an automated high-throughput process known as systematic evolution of ligands by exponential enrichment (SELEX)[79]. More than 15 years after the initial description of this method, the first aptamer-based drug pegaptanib (Macugen; Eyetech Pharmaceuticals/Pfizer, New York) a PEG-ylated anti–vascular endothelial growth factor (VEGF) aptamer for the treatment of wet age-related macular degeneration, was approved by the FDA[80]. The optimized and heavily modified

Box 1 Drugs and druggability

For the pharmaceutical industry, the ideal drug is a small molecule featuring high potency, selectivity, few adverse effects, low production costs and administration in a single daily oral dose. However, identifying compounds with these properties is challenging, and drug companies have been confronted with an ever-growing drug attrition rate[97]. During the drug discovery process, molecules that fulfill a set of molecular guidelines for oral bioavailability, known as the "Rule of 5"[98] (RO5; e.g., a molecular weight below 500 Da or a partition coefficient [logP] below 5), are referred to as "drug-like" compounds. Similarly, this concept can be transferred to the targets of therapeutic intervention. Druggability refers to disease-related proteins that have the potential to be modulated by drug-like compounds[97]. Several attempts have been made to predict the druggability of protein families and to identify promising drug targets. Using the "druggable genome" approach, the targets of currently marketed drugs have been analyzed and mapped to protein families, assuming persistent druggability within the family[99]. Enzymes are the predominant class (nearly 50% of the total), followed by G-protein-coupled receptors (30%) and ion channels (7%). Within the complement cascade, the only targets with acceptable druggability potential are therefore the serine proteases and the anaphylatoxin receptors. Considering the large post-translational variability of protein targets and the increasing market for biopharmaceuticals, expansions of this model, such as the "pharmaceutically tractable genome," have recently been suggested[100].

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Box 2 Supporting the niches—the Orphan Drug Act

Development of a new drug compound is a challenging and expensive endeavor. The cost for a newly marketed drug is currently estimated to be in the range of $500 million to more than $2,000 million[101]. It is therefore understandable that pharmaceutical companies focus primarily on drugs to treat major diseases with a large market potential (that is, "blockbuster drugs"). This focus often leaves a painful therapeutic gap for rare diseases, niche markets or biopharmaceuticals with patent limitations (e.g., natural proteins). Compounds that are unprofitable to develop are usually referred to as "orphan drugs"[102]. In an effort to encourage the development of such drugs, several drug regulation authorities, including the US Food and Drug Administration (FDA), have initiated special programs such as the Orphan Drug Act (ODA) of 1983 (ref. 103). Designation of orphan drug status is coupled to a series of financial incentives, the most important of which is marketing exclusivity for 7 years after drug approval. In addition, orphan product development grants sponsor clinical trials and close the gap between basic research and clinical development. Undeniably, the ODA is one of the most successful recent United States legislative actions and has had a major impact on the drug market. Twenty years after its enactment, over 1,000 drugs had been accorded orphan drug status, and more than 200 had been approved for marketing, thereby improving the clinical situation of an estimated 11 million patients in the United States alone[102]. Meanwhile, these numbers have grown to 1,750 designated and 315 marketed orphan drugs (as of October 4, 2007, http://www.fda.gov/orphan/designat/list.htm). Similar initiatives also exist in other countries and regions (e.g., at the European Medicines Agency). In the complement field, eculizumab, for the treatment of paroxysmal nocturnal hemoglobinuria, and C1 inhibitor concentrate, for treatment of hereditary angioedema, are covered by the ODA. For more information, see http://www.fda.gov/orphan/.

form of the anti-C5 RNA aptamer (ARC1905; Archemix, Cambridge, MA, USA) features a subnanomolar binding affinity for human C5. It provides complete blockage of downstream complement activation by inhibiting the cleavage of C5 into C5a and C5b. A 40-kDa PEG group has been added to the 5′ end to improve its pharmacokinetic properties. Although ARC1905 was initially developed for use during coronary artery bypass graft surgery, the recent failure of pexelizumab for this indication has had a direct impact on the fate of ARC1905. Like compstatin (POT-4) and Macugen, the compound is now positioned as an intravitreal treatment option for wet and dry age-related macular degeneration and is awaiting clinical trials (P. Bouchard, Archemix Corp., personal communication).

Anaphylatoxin receptor antagonists. The proinflammatory activity of the anaphylatoxin C5a is the driving force behind many complement-associated disorders. After its liberation from C5, this hormone-like glycoprotein binds to two high-affinity receptors, the C5a receptor (C5aR) and C5L2 (**Fig. 2**)[81]. Most of the proinflammatory signaling seems to be induced by C5aR, a G-protein-coupled receptor that is expressed on numerous myeloid (e.g., neutrophils, macrophages) and nonmyeloid cells. Despite its similar structure and affinity for C5a, the C5L2 receptor produces a completely different signaling pattern. In plasma, the activity of C5a is controlled through the removal of the C-terminal arginyl residue by the plasma enzyme carboxypeptidase N. The resulting $C5a^{desArg}$ binds poorly to C5aR but with a high affinity to C5L2. Earlier reports about binding of C3a and $C3a^{desArg}$ to C5L2 have recently been questioned and require further investigation[81]. The differential binding pattern, as well as reports of the anti-inflammatory activity mediated by C5L2, has led to the hypothesis that the regulation of C5a involves a sophisticated feedback mechanism[20,64]. However, other studies have reported a proinflammatory or C5aR-supporting role for C5L2 (ref. 82), leaving its exact function a matter of scientific debate.

Selective inhibition of the binding of C5a to its receptors offers a very promising opportunity for dampening the inflammatory response without depleting the defensive potential of complement[64,83,84]. The macromolecular biopharmaceuticals we have previously discussed either inhibit C5a generation (anti-C5 antibodies and aptamers) or neutralize the C5a-C5aR interaction by shielding the relevant binding site. C5aR antagonists, on the other hand, are designed to bind to the receptor with high affinity without inducing any signaling activity (**Fig. 2b**). Because only one of the two proposed binding sites on C5aR has to be targeted for this purpose[81,84], these antagonists are expected to be rather small. Simultaneous efforts by many companies have focused on reducing the functional fragment of C5a to a C-terminal hexapeptide, while replacing individual residues to shift its activity from agonistic to antagonistic. Cyclization of these hexapeptides not only allows them to be locked into the desired turn-conformation but also gives them higher stability with regard to proteolytic degradation[84].

The most promising candidate to emerge, the cyclic peptidomimetic PMX-53, is currently under development by Peptech[85]. With a molecular size under 1 kDa and a high oral bioavailability, PMX-53 has many of the desired features of a designed drug. However, its short half-life of only 70 min may mean a requirement for frequent, if not continuous, administration. Although the drug showed high stability in blood, gastric and intestinal contents, it was rapidly degraded by proteolysis in intestinal mucosa washings. In a phase 1a/2b trial in 21 patients with rheumatoid arthritis, PMX-53 was orally administered and showed positive trends in many disease measures. An equally positive result was observed in a phase 1b/2a trial of topical application of the drug in ten people with psoriasis. In both studies, PMX-53 was found to be safe and well-tolerated[85]. Despite these encouraging results, the short half-life and rapid degradation in intestinal mucosa of this drug may severely limit its use. It remains to be seen if Peptech will further develop PMX-53 or initiate new trials with PMX-205, a similar yet more hydrophobic compound that shows high potency but a much lower susceptibility to proteolysis than that of PMX-53 (refs. 86,87).

Given the current uncertainty about the role of the C5L2 receptor, a critical evaluation of the binding specificity may be an important requirement for the development of successful C5aR antagonists. Several companies seem to have promising candidates in preclinical testing. For example, Jerini has a series of peptidomimetics and small organic molecules (JPE-1375, JSM-7717) for the treatment of inflammatory, renal and ocular diseases in preclinical stages (http://www.jerini.com/).

Other concepts. Whereas most of the previously discussed concepts are designed to inhibit or decrease complement activation, one compound is intended to boost low complement activity. Approximately 30% of the human population shows low plasma levels of MBL, making them more susceptible to infections under immunosuppressive conditions. Recombinant human MBL (rhMBL; Enzon, Bridgewater NJ, USA) restores the activity of the lectin pathway and has been found to be safe and well tolerated[88]. It is currently being tested in phase 1b trials as replacement therapy in people with multiple myeloma who are deficient

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in MBL and undergoing high-dose chemotherapy and stem cell transplants, and in MBL-deficient people undergoing liver transplantation. Additional approaches such as quenching of exposed thioester groups of C3b (thioester inhibitors)[17] or inhibition of the C1q-IgG interaction by anionic small molecules (e.g., steroid/triterpenoid sulfates) have been described[31,89] but have not appeared in preclinical settings thus far.

Perspectives and outlook

As is true for complement research itself, the quest for complement-directed therapeutics has made dramatic changes in direction and encountered difficult challenges; initial euphoria has given way to a more realistic view of strategies and aims. Whereas some first-generation approaches of complement drug discovery have either failed (e.g., small-molecule serine protease inhibitors) or are severely struggling (e.g., soluble regulators), there are many promising concepts on the horizon that represent a second generation of complement drugs. Current indications for complement drugs, such as HAE or PNH, certainly represent niche markets far away from blockbuster areas. Both eculizumab and the C1-INH concentrates are protected under the Orphan Drug Act (**Box 2**), and their approval is an important step in establishing the concept and accumulating clinical experience before expanding their indications.

Undeniably, the unsatisfactory results obtained for TP10 and pexelizumab have left a bitter taste and may have had a negative impact on the field. These disappointments underscore the limitations inherent in broadly applying complement-specific drugs in inflammatory diseases. Many of the compounds that have been discontinued were designed to target multifaceted disorders such as I/R injury. In these disorders, many parallel mechanisms are involved, and the influence of complement is still a matter of debate. For such complex indications, the key to success may be to use complement drugs not as single therapies but (in consonance with their name) as complementary therapies. Clearly, more basic research and additional tests are required in this context. In cases in which clear complement targets can be identified (as in HAE or PNH), complement therapeutics have already been successfully applied.

Several factors are expected to boost the development of complement therapeutics. In particular, our accumulating knowledge about the exact role of complement in various diseases will greatly facilitate the identification of relevant targets. Even more importantly, crystal structures of a number of crucial complement components (e.g., C3 (ref. 90), C3b[91,92], C3c[90], C3d[93], factor B[94], factor D[95]) have recently been solved and are available for structure-based design. Genentech has reported a co-crystal structure of C3b and C3c with the extracellular domain of the macrophage complement receptor CRIg[92]. The same group recently demonstrated that soluble murine CRIg selectively inhibits the alternative pathway by competing with C5 for convertase binding, and that it was effective in mouse models of arthritis[96]. Its high selectivity for the alternative pathway and the availability of a detailed crystal structure make CRIg an attractive lead structure and render its further clinical development highly likely. Along with the continuous development of established approaches, the quest for complement therapeutics may get help from an unexpected source: human pathogens. *Staphylococcus aureus* (*S. aureus*) alone offers several interesting candidates. Among them, the extracellular fibrinogen-binding protein[28] and the staphylococcal complement inhibitor[30] of *S. aureus* have been reported to inhibit the conversion of C3 to C3a and C3b. An understanding of their interaction sites and mechanisms could be applied to developing either potent complement inhibitors or new antibiotics targeting *S. aureus*. In addition, the chemotaxis inhibitory protein of *S. aureus*[29] has been found to antagonize C5aR and may therefore serve as a lead structure for novel antagonists.

In many aspects, complement drug discovery illustrates general trends that are observed in the pharmaceutical industry. Limitations in the development of small compounds (e.g., lack of specificity or inability to disrupt protein-protein interactions) and improved technology and safety in the production of natural macromolecules has led to a growing market for these biopharmaceuticals. An analysis of the compounds indicates that they almost exclusively represent large proteins and nucleotides, or that they have been developed from peptides. Furthermore, major pharmaceutical companies tend to outsource early drug discovery phases to academic institutions and small startup companies. Partnerships with major pharmaceutical companies are usually only sealed after clinical trials have begun (often after phase I/2). Indeed, many of the promising complement drug candidates (e.g., compstatin and PMX-53) were initially developed in the academic field and further developed by biotechnology companies. Such partnerships have already been established in the case of ofatumumab (Genmab/Glaxo), pexelizumab (Alexion/Procter & Gamble, Cincinnati, OH, USA) and neutrazumab (G2 Therapies/Novo Nordisk).

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

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(71) Applicants *(for all designated States except US)*: **ALEXION PHARMACEUTICALS, INC.** [US/US]; 352 Knotter Drive, Cheshire, CT 06410 (US). **THE UNIVERSITY OF WESTERN ONTARIO** [CA/CA]; 1151 Richmond Street North, Stevenson-Lawson Building, Room 328, London, Ontario N6A 5B8 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROTHER, Russell, P.** [US/US]; 25 Blue Trail Drive, Prospect, CT 06712 (US). **WANG, Hao** [CA/CA]; 480 Sandybrook Drive, London, Ontario N5X 4H2 (CA). **ZHONG, Zhen** [CA/CA]; 60 Nathaniel Court, London, Ontario N5X 2N5 (CA).

(74) Agents: **WU, Xuqiong** et al.; Fish & Neave IP Group of, Ropes & Gray LLP, One International Place, Boston, MA 02110-2624 (US).

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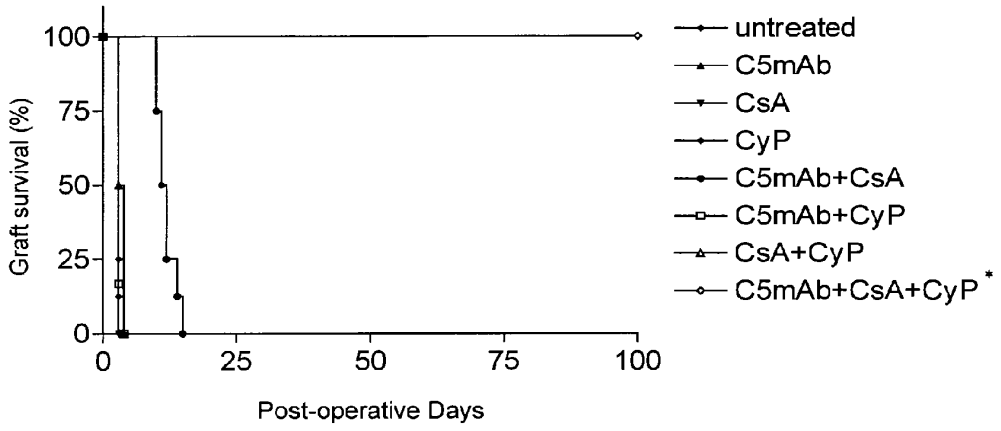

(57) Abstract: Methods of prolonging survival of allotransplanted cells, tissues or organs are presented. These methods are directed to administering to the allotransplant recipient an inhibitor of complement activity together with one or more immunosuppressants. The inhibitor of complement activity is administered chronically. These methods have been determined to aid in preventing chronic rejection of allografts. These methods can additionally be used in cases in which the recipient has been presensitized to the allograft or in which there is an ABO mismatch between the allograft and the recipient.

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PROLONGATION OF SURVIVAL OF AN ALLOGRAFT BY INHIBITING COMPLEMENT ACTIVITY

RELATED APPLICATIONS

This application claims the benefit of and priority to U.S. Provisional Patent Application No. 60/571,444, filed May 14, 2004, the disclosure of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The present disclosure relates to methods for prolonging survival of an allograft in a mammal. In particular, the present disclosure relates to prolonging survival of an allograft by administering an inhibitor of complement or terminal complement formation, especially an inhibitor of complement C5 cleavage, in addition to one or more drugs that are immunosuppressant.

BACKGROUND

Organ transplantation is the preferred treatment for most patients with chronic organ failure. Although transplantation of kidney, liver, lung, and heart offers an excellent opportunity for rehabilitation as recipients return to a more normal lifestyle, it is limited by the medical/surgical suitability of potential recipients, an increasing shortage of donors, and premature failure of transplanted organ function.

Transplantation of cells, tissues and organs has become very common and is often a life-saving procedure. Organ transplantation is the preferred treatment for most patients with chronic organ failure. Despite great improvement in treatments to inhibit rejection, rejection continues to be the single largest impediment to successful organ transplantation. Rejection includes not only acute rejection but also chronic rejection. One-year survival rates for transplanted kidneys average 88.3% with kidneys from deceased donors and 94.4% with kidneys received from living donors. The corresponding five year survival rates for the transplanted kidneys are 63.3% and 76.5% (OPTN/SRTR Annual Report, 2002). For livers the one year survival rates are 80.2% and 76.5% for livers from deceased and living donors, respectively. The corresponding five year liver graft survival rates are 63.5% and 73.0% (OPTN/SRTR Annual Report, 2002). The use of immunosuppressant drugs, especially cyclosporin A and more recently tacrolimus, has dramatically improved the success rate of organ transplantation especially by preventing acute rejection. But as the numbers above

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show, there is still a need to improve the success rates, both short-term and especially long-term. As seen from the above numbers for kidney and liver transplants, the five year failure rates for these transplanted organs are on the order of 25-35%. In the year 2001 alone there were more than 23,000 patients who received an organ transplant of which approximately 19,000 were kidney or liver (OPTN/SRTR Annual Report, 2002). For this one year of transplants alone, with present techniques it can be expected that approximately 5,000-6,000 of these transplanted kidneys and livers will fail within 5 years. These numbers do not even include other transplanted organs or transplanted tissues or cells such as bone marrow.

There are multiple types of transplants. These are described in Abbas et al., 2000. A graft transplanted from one individual to the same individual is called an autologous graft or autograft. A graft transplanted between two genetically identical or syngeneic individual is called a syngeneic graft. A graft transplanted between two genetically different individuals of the same species is called an allogeneic graft or allograft. A graft transplanted between individuals of different species is called a xenogeneic graft or xenograft. The molecules that are recognized as foreign on allografts are called alloantigens and those on xenografts are called xenoantigens. The lymphocytes or antibodies that react with alloantigens or xenoantigens are described as being alloreactive or xenoreactive, respectively.

Currently more than 40,000 kidney, heart, lung, liver and pancreas transplants are performed in the United States each year (Abbas et al., 2000). Other possible transplants include, but are not limited to, vascular tissue, eye, cornea, lens, skin, bone marrow, muscle, connective tissue, gastrointestinal tissue, nervous tissue, bone, stem cells, islets, cartilage, hepatocytes, and hematopoietic cells. Unfortunately, there are many more candidates for a transplant than there are donors. To overcome this shortage, a major effort is being made to learn how to use xenografts. While progress is being made in this field, the fact is that at present most transplants are allografts. An allogeneic transplant, while presently being more likely to be successful than a xenogeneic transplant, must surmount numerous obstacles to be successful. There are several types of immunological attacks made by the recipient against the donor organ which can lead to rejection of the allograft. These include hyperacute rejection, acute vascular rejection (including accelerated humoral rejection and de novo acute humoral rejection), and chronic rejection. Rejection is normally a result of T-cell mediated or humoral antibody attack, but may include additional secondary factors such as the effects of complement and cytokines.

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An ever growing gap between the number of patients requiring organ transplantation and the number of donor organs available has become a major problem throughout the world. Park et al., 2003. Individuals who have developed anti-HLA antibodies are said to be immunized or sensitized. Gloor, 2005. HLA sensitization is the major barrier to optimal utilization of organs from living donors in clinical transplantation (Warren et al., 2004) due to the development of severe antibody-mediated rejection (ABMR). For example, more than 50% of all individuals awaiting kidney transplantation are presensitized patients (Glotz et al., 2002) who have elevated levels of broadly reactive alloantibodies, resulting from multiple transfusions, prior failed allografts, or pregnancy (Kupiec-Weglinski, 1996). The role of ABMR is currently one of the most dynamic areas of study in transplantation, due to recognition that this type of rejection can lead to either acute or chronic loss of allograft function. Mehra et al., 2003. Numerous cases of ABMR, including hyperacute rejection (HAR) or accelerated humoral rejection (ACHR), have been reported that are characterized by acute allograft injury that is resistant to potent anti-T cell therapy, the detection of circulating donor specific antibodies, and the deposition of complement components in the graft. ABMR with elevated circulating alloantibodies and complement activation that occurs in 20-30% of acute rejection cases has a poorer prognosis than cellular rejection. Mauiyyedi et al., 2002.

Highly presensitized patients, who exhibit high levels of alloantibodies, usually suffer an immediate and aggressive HAR. In clinical practice, with great efforts and significant advances in technology, HAR is avoided by obtaining a pretransplant lymphocytotoxic cross-match to identify sensitized patients with antibodies specific for donor HLA antigens. However, circulating antibodies against donor HLA or other non-MHC endothelial antigens may also be responsible for a delayed form of acute humoral rejection, which is associated with an increased incidence of graft loss. Collins et al., 1999. Therefore, development of a novel presensitized animal model to mimic ABMR in clinical settings would be beneficial to studies on the mechanism, and to the much needed progress in the management of allograft rejection in presensitized hosts.

Some highly presensitized patients can benefit from intervention programs such as immunoadsorption (Palmer et al.,1989; Ross et al., 1993; Kriaa et al., 1995), plasmapheresis and intravenous immunoglobulin (Sonnenday et al., 2002; Rocha et al., 2003), that have been designed and implemented to temporarily eliminate anti-donor antibodies. However, in addition to their benefits, the aforementioned therapies carry with them numerous drawbacks

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as some individuals are less susceptible to their effects (Kriaa et al., 1995; Hakim et al., 1990; Glotz et al., 1993; Tyan et al., 1994); they are extremely expensive, time-consuming, and risky (Salama et al., 2001). Moreover, the transient and variable effect of these protocols has limited their impact. Glotz et al., 2002; Kupin et al., 1991; Schweitzer et al., 2000. Therefore, developing novel strategies to reduce the risk and cost in prevention of ABMR would be beneficial to presensitized recipients receiving an allograft.

SUMMARY

Accordingly, methods of prolonging survival of transplanted cells, tissues or organs are provided. In particular, methods of prolonging survival of allotransplanted cells, tissues or organs are provided. These methods are directed to using one or more immunosuppressants in addition to an inhibitor of complement activity. Use of one or more immunosuppressants and an inhibitor of complement activity in the manufacture of one or more medicaments or medicament packages is also provided. Such medicaments or medicament packages are useful in prolonging survival of an allograft in a subject mammal.

In certain embodiments, the inhibition of complement activity is effected by chronic administration of a drug directed against complement C5. A preferred drug that inhibits complement activity is an antibody specific to one or more components of complement, for example, C5. In certain preferred embodiments, the antibody inhibits the cleavage of C5 and thereby inhibits the formation of both C5a and C5b-9. The antibody may be, e.g., a monoclonal antibody, a chimeric antibody (e.g., a humanized antibody), an antibody fragment (e.g., Fab), a single chain antibody, an Fv, or a domain antibody. The recipient is also treated with one or more immunosuppressive drugs, for example, cyclosporin A.

In certain embodiments, either an MHC mismatched recipient (i.e., a mammalian recipient of an MHC mismatched allograft), a presensitized recipient or an ABO mismatched recipient (i.e., a mammalian recipient of an AMB mismatched allograft) is treated. In this model, the recipient is again chronically treated with a complement inhibitor, preferably an anti-C5 monoclonal antibody, together with immunosuppressive drugs, preferably a chronic administration of cyclosporin A and a short-term administration of cyclophosphamide. This triple therapy results in extended graft survival in the presensitized allotransplant recipient.

The present disclosure also provides methods of prolonging survival of an allograft in a mammalian recipient by administering to the recipient agents that modulate the level and/or ratio of subclasses and/or isotypes of anti-donor immunoglobulins (Ig) in the recipient. In

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certain embodiments, an agent that reduces the level of anti-donor IgG1 in the recipient is preferred. In certain embodiments, an agent that increases the level of anti-donor IgG2a and/or IgG2b in the recipient is preferred. In certain embodiments, an agent that reduces the ratio of anti-donor IgG1/anti-donor IgG2a or IgG2b in the recipient is preferred.

The present disclosure also provides a method of prolonging survival of an allograft in a second mammalian recipient using an allograft that has been accommodated in a first mammalian recipient (i.e., the allograft has prolonged survival in the first recipient). The present disclosure further provides an allograft that is resistant to anti-donor antibodies in a mammalian recipient, and the allograft is prepared from a first recipient that has accommodated the allograft. In preferred embodiments, the first recipient has accommodated the allograft by receiving a treatment as described herein, such a triple therapy treatment involving administering to the first recipient a drug that inhibits complement activity and two immunosuppressive agents.

Further provided are pharmaceutical packages. A pharmaceutical package of the present disclosure may comprise a drug that inhibits complement activity and at least one immunosuppressive agent. The pharmaceutical package may further comprise a label for chronic administration. The pharmaceutical package may also comprise a label for self-administration by a patient, for example, a recipient of a transplant graft, or instructions for a caretaker of a recipient of a transplant graft. In certain embodiments, the drug and the agent in the pharmaceutical package are in a formulation or separate formulations that are suitable for chronic administration and/or self-administration.

The present disclosure also provides lyophilized formulations and formulations suitable for injection. Certain embodiments provide a lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant. In preferred embodiments, the antibody formulation is suitable for chronic administration, for example, the antibody formulation is stable. Alternative embodiments provide an injection system comprising a syringe; the syringe comprises a cartridge containing an antibody that inhibits complement activity and is in a formulation suitable for injection.

An antibody employed in various embodiments of the present disclosure preferably inhibits the formation of terminal complement or C5a. In certain embodiments, antibody inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment. The whole antibody or antibody fragment may be a human, humanized,

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chimerized or deimmunized antibody or antibody fragment. In certain embodiments, the whole antibody or antibody fragment may inhibit cleavage of complement C5. In certain embodiments, the antibody fragment is a Fab, an F(ab')2, an Fv, a domain antibody, or a single-chain antibody. In preferred embodiments, the antibody fragment is pexelizumab. In alternative preferred embodiments, the whole antibody is eculizumab.

In certain embodiments, a drug, such as an antibody, that inhibits complement activity is present in unit dosage form, which can be particularly suitable for self-administration. Similarly, an immunosuppressive agent of the present disclosure may also be present in unit dosage form.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D show anti-donor antibody levels in presensitized versus unsensitized recipients under different treatments.

Figures 2A and 2B show comparison between triple therapy using anti-C5 antibody, CsA and CyP in presensitized allograft recipients and combination therapy using only anti-C5 antibody and CsA in presensitized allograft recipients. Figure 2A compares heart-allograft survival in various recipients under different treatments as indicated. Figure 2B shows histology and immunohistology, for example, for lymphocyte infiltration in heart allografts of recipients in different groups.

Figure 3 shows blocked terminal complement activity by anti-C5 antibody as compared to immunosuppressive agents.

Figures 4A-4D compare levels of anti-donor antibodies in presensitized recipients of allografts under monotherapy with anti-C5 antibody alone, double combination therapy with anti-C5 antibody and CsA, and triple combination therapy with anti-C5 antibody, CsA and CyP.

Figures 5A and 5B show change of ratios of IgG isotypes in allograft recipients that were untreated or under different treatments.

Figure 6 shows high-level expression of Bcl-2 and Bcl-xl proteins in long-term surviving heart grafts as compared to heart grafts of untreated animals.

Figure 7 shows improved second transplantation (re-transplantation) of an accommodated graft from a first transplantation recipient.

Figure 8 shows results from re-transplantation experiments.

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Figure 9 shows results from re-transplantation experiments.

DETAILED DESCRIPTION

Overview: Rejection of Transplants or Grafts

Hyperacute rejection occurs within minutes to hours after transplant and is due to preformed antibodies to the transplanted tissue antigens. It is characterized by hemorrhage and thrombotic occlusion of the graft vasculature. The binding of antibody to endothelium activates complement, and antibody and complement induce a number of changes in the graft endothelium that promote intravascular thrombosis and lead to vascular occlusion, the result being that the grafted organ suffers irreversible ischemic damage (Abbas et al., 2000). Hyperacute rejection is often mediated by preexisting IgM alloantibodies, e.g., those directed against the ABO blood group antigens expressed on red blood cells. This type of rejection, mediated by natural antibodies, is the main reason for rejection of xenotransplants. Hyperacute rejection due to natural IgM antibodies is no longer a major problem with allografts because allografts are usually selected to match the donor and recipient ABO type. Hyperacute rejection of an ABO matched allograft may still occur, usually mediated by IgG antibodies directed against protein alloantigens, such as foreign MHC molecules, or against less well defined alloantigens expressed on vascular endothelial cells. Such antibodies may arise as a result of prior exposure to alloantigens through blood transfusion, prior transplantation, or multiple pregnancies (this prior exposure being referred to as "presensitization"). Abbas et al., 2000.

Acute rejection is a process of vascular and parenchymal injury mediated by T cells, macrophages, and antibodies that usually begins after the first week of transplantation. Abbas et al., 2001. T lymphocytes play a central role in acute rejection by responding to alloantigens, including MHC molecules, present on vascular endothelial and parenchymal cells. The activated T cells cause direct lysis of graft cells or produce cytokines that recruit and activate inflammatory cells, which cause necrosis. Both $CD4^+$ and $CD8^+$ cells may contribute to acute rejection. The destruction of allogeneic cells in a graft is highly specific and a hallmark of $CD8^+$ cytotoxic T lymphocyte killing. Abbas et al., 2000. $CD4^+$ T cells may be important in mediating acute graft rejection by secreting cytokines and inducing delayed-type hypersensitivity-like reactions in grafts, with some evidence available that indicates that $CD4^+$ T cells are sufficient to mediate acute rejection. Abbas et al., 2000. Antibodies can also mediate acute rejection after a graft recipient mounts a humoral immune

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response to vessel wall antigens and the antibodies that are produced bind to the vessel wall and activate complement. Abbas et al., 2000.

Chronic rejection is characterized by fibrosis with loss of normal organ structures occurring over a prolonged period. The pathogenesis of chronic rejection is less well understood than that of acute rejection. Graft arterial occlusion may occur as a result of the proliferation of intimal smooth muscle cells (Abbas et al., 2000). This process is called accelerated or graft arteriosclerosis and can develop in any vascularized organ transplant within 6 months to a year after transplantation.

For a transplant to be successful, the several modes of rejection must be overcome. Multiple approaches are utilized in preventing rejection. This may require administration of immunosuppressants, often several types to prevent the various modes of attack, e.g., inhibition of T-cell attack, antibodies, and cytokine and complement effects. Prescreening of donors to match them with recipients is also a major factor in preventing rejection, especially in preventing hyperacute rejection. Immunoadsorption of anti-HLA antibodies prior to grafting may reduce hyperacute rejection. Prior to transplantation the recipient or host may be administered anti-T cell reagents, e.g., the monoclonal antibody OKT3, Anti-Thymocyte Globulin (ATG), cyclosporin A, or tacrolimus (FK 506). Additionally, glucocorticoids and/or azathioprine may be administered to the host prior to transplant. Drugs used to aid in preventing transplant rejection include, but are not limited to, ATG or ALG, OKT3, daclizumab, basiliximab, corticosteroids, 15-deoxyspergualin, cyclosporins, tacrolimus, azathioprine, methotrexate, mycophenolate mofetil, 6-mercaptopurine, bredinin, brequinar, leflunamide, cyclophosphamide, sirolimus, anti-CD4 monoclonal antibodies, CTLA4-Ig, anti-CD154 monoclonal antibodies, anti-LFA1 monoclonal antibodies, anti-LFA-3 monoclonal antibodies, anti-CD2 monoclonal antibodies, and anti-CD45.

Allografts are rejected in part by the activation of T cells. The transplant recipient mounts a rejection response following $CD4^+$ T cell recognition of foreign antigens in the allograft. These antigens are encoded by the major histocompatibility complex (MHC). There are both Class I and Class II MHC molecules. In humans the class I MHC molecules are HLA-A, HLA-B, and HLA-C. The class II MHC molecules in humans are called HLA-DR, HLA-DQ and HLA-DP. In mice the class I MHC molecules are H-2K, H-2D and H-2L and the class II MHC molecules are I-A and I-E. When $CD4^+$ T cells bind the foreign MHC antigens they are activated and undergo clonal proliferation. The activated T cells secrete cytokines which aid in activating monocytes/macrophages, B cells and cytotoxic $CD8^+$ T

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cells. The activated monocytes/macrophages release agents which result in tissue damage, the B cells produce alloantibodies which lead to complement mediated tissue destruction, and the $CD8^+$ T cells kill graft cells in an antigen-specific manner through induction of apoptosis and cell lysis.

Immunosuppressive Agents

The numerous drugs utilized to delay graft rejection (i.e., to prolong their survival) work in a variety of ways. Immunosuppressive agents are widely used. See Stepkowski, 2000, for a review of the mechanism of action of several immunosuppressive drugs. Cyclosporin A is one of the most widely used immunosuppressive drugs for inhibiting graft rejection. It is an inhibitor of interleukin-2 or IL-2 (it prevents mRNA transcription of interleukin-2). More directly, cyclosporin inhibits calcineurin activation that normally occurs upon T cell receptor stimulation. Calcineurin dephosphorylates NFAT (nuclear factor of activated T cells) enabling it to enter the nucleus and bind to interleukin-2 promoter. By blocking this process, cyclosporin A inhibits the activation of the $CD4^+$ T cells and the resulting cascade of events which would otherwise occur. Tacrolimus is another immunosuppressant that acts by inhibiting the production of interleukin-2.

Rapamycin (Sirolimus), SDZ RAD, and interleukin-2 receptor blockers are drugs that inhibit the action of interleukin-2 and therefore prevent the cascade of events described above.

Inhibitors of purine or pyrimidine biosynthesis are also used to inhibit graft rejection. These prevent DNA synthesis and thereby inhibit cell division including the ability of T cells to divide. The result is the inhibition of T cell activity by preventing the formation of new T cells. Inhibitors of purine synthesis include azathioprine, methotrexate, mycophenolate mofetil (MMF) and mizoribine (bredinin). Inhibitors of pyrimidine synthesis include brequinar sodium and leflunomide. Cyclophosphamide is an inhibitor of both purine and pyrimidine synthesis.

Yet another method for inhibiting T cell activation is to treat the recipient with antibodies to T cells. OKT3 is a murine monoclonal antibody against CD3 which is part of the T cell receptor. This antibody inhibits the T cell receptor and suppresses T cell activation.

Numerous other drugs and methods for delaying allotransplant rejection are known to and used by those of skill in the art. One approach has been to deplete T cells, e.g., by irradiation. This has often been used in bone marrow transplants, especially if there is a

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partial mismatch of major HLA. Administration to the recipient of an inhibitor (blocker) of the CD40 ligand-CD40 interaction and/or a blocker of the CD28-B7 interaction has been used (U.S. Patent 6,280,957). Published PCT patent application WO 01/37860 teaches the administration of an anti-CD3 monoclonal antibody and IL-5 to inhibit the Th1 immune response. Published PCT patent application WO 00/27421 teaches a method for prophylaxis or treatment of corneal transplant rejection by administering a tumor necrosis factor-α antagonist. Glotz et al. (2002) show that administration of intravenous immunoglobulins (IVIg) can induce a profound and sustained decrease in the titers of anti-HLA antibodies thereby allowing a transplant of an HLA-mismatched organ. Similar protocols have included plasma exchanges (Taube et al., 1984) or immunoadsorption techniques coupled to immunosuppressive agents (Hiesse et al., 1992) or a combination of these (Montgomery et al., 2000). Changelian et al. (2003) teach a model in which immunosuppression is caused by an oral inhibitor of Janus kinase 3 (JAK3) which is an enzyme necessary for the proper signaling of cytokine receptors which use the common gamma chain (γc) (Interleukins-2, -4, -7, -9, -15, -21), the result being an inhibition of T cell activation. Antisense nucleic acids against ICAM-1 have been used alone or in combination with a monoclonal antibody specific for leukocyte-function associated antigen 1 (LFA-1) in a study of heart allograft transplantation (Stepkowski, 2000). Similarly, an anti-ICAM-1 antibody has been used in combination with anti-LFA-1 antibody to treat heart allografts (Stepkowski, 2000). Antisense oligonucleotides have additionally been used in conjunction with cyclosporin in rat heart or kidney allograft models, resulting in a synergistic effect to prolong the survival of the grafts (Stepkowski, 2000). Chronic transplant rejection has been treated by administering an antagonist of TGF-β which is a cytokine involved in differentiation, proliferation and apoptosis (U.S. Patent Application Publication US 2003/0180301).

Complement and Transplant/Graft Rejection

The role of complement in transplant rejection is well known. This is especially true in the case of xenotransplantation, but complement also plays a role in allotransplant rejection. For review, see Platt and Saadi, 1999. One aspect of complement's role is that ischemia-reperfusion injury may occur at the time that an organ graft is reperfused with the blood of the recipient. Complement may also cause some manifestations of allograft rejection.

The complement system is described in detail in U.S. Patent 6,355,245. The complement system acts in conjunction with other immunological systems of the body to

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defend against intrusion of cellular and viral pathogens. There are at least 25 complement proteins, which are found as a complex collection of plasma proteins and membrane cofactors. The plasma proteins make up about 10% of the globulins in vertebrate serum. Complement components achieve their immune defensive functions by interacting in a series of intricate but precise enzymatic cleavage and membrane binding events. The resulting complement cascade leads to the production of products with opsonic, immunoregulatory, and lytic functions.

The complement cascade progresses via the classical pathway or the alternative pathway. These pathways share many components and, while they differ in their initial steps, they converge and share the same "terminal complement" components (C5 through C9) responsible for the activation and destruction of target cells.

The classical complement pathway is typically initiated by antibody recognition of and binding to an antigenic site on a target cell. The alternative pathway is usually antibody independent and can be initiated by certain molecules on pathogen surfaces. Both pathways converge at the point where complement component C3 is cleaved by an active protease (which is different in each pathway) to yield C3a and C3b. Other pathways activating complement attack can act later in the sequence of events leading to various aspects of complement function.

C3a is an anaphylatoxin. C3b binds to bacterial and other cells, as well as to certain viruses and immune complexes, and tags them for removal from the circulation. C3b in this role is known as opsonin. The opsonic function of C3b is considered to be the most important anti-infective action of the complement system. Patients with genetic lesions that block C3b function are prone to infection by a broad variety of pathogenic organisms, while patients with lesions later in the complement cascade sequence, i.e., patients with lesions that block C5 functions, are found to be more prone only to Neisseria infection, and then only somewhat more prone (Fearon, 1983).

C3b also forms a complex with other components unique to each pathway to form classical or alternative C5 convertase, which cleaves C5 into C5a and C5b. C3 is thus regarded as the central protein in the complement reaction sequence since it is essential to both the alternative and classical pathways (Wurzner et al., 1991). This property of C3b is regulated by the serum protease Factor I, which acts on C3b to produce iC3b. While still functional as opsonin, iC3b cannot form an active C5 convertase.

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C5 is a 190 kDa beta globulin found in normal serum at approximately 75 μg/mL (0.4 μM). C5 is glycosylated, with about 1.5-3 percent of its mass attributed to carbohydrate. Mature C5 is a heterodimer of a 999 amino acid 115 kDa alpha chain that is disulfide linked to a 656 amino acid 75 kDa beta chain. C5 is synthesized as a single chain precursor protein product of a single copy gene (Haviland et al., 1991). The cDNA sequence of the transcript of this gene predicts a secreted pro-C5 precursor of 1659 amino acids along with an 18 amino acid leader sequence.

The pro-C5 precursor is cleaved after amino acid 655 and 659, to yield the beta chain as an amino terminal fragment (amino acid residues +1 to 655) and the alpha chain as a carboxyl terminal fragment (amino acid residues 660 to 1658), with four amino acids deleted between the two.

C5a is cleaved from the alpha chain of C5 by either alternative or classical C5 convertase as an amino terminal fragment comprising the first 74 amino acids of the alpha chain (i.e., amino acid residues 660-733). Approximately 20 percent of the 11 kDa mass of C5a is attributed to carbohydrate. The cleavage site for convertase action is at or immediately adjacent to amino acid residue 733. A compound that would bind at or adjacent to this cleavage site would have the potential to block access of the C5 convertase enzymes to the cleavage site and thereby act as a complement inhibitor.

C5 can also be activated by means other than C5 convertase activity. Limited trypsin digestion (Minta and Man, 1977; Wetsel and Kolb, 1982) and acid treatment (Yamamoto and Gewurz, 1978; Vogt et al., 1989) can also cleave C5 and produce active C5b.

C5a is another anaphylatoxin. C5b combines with C6, C7, and C8 to form the C5b-8 complex at the surface of the target cell. Upon binding of several C9 molecules, the membrane attack complex (MAC, C5b-9, terminal complement complex-TCC) is formed. When sufficient numbers of MACs insert into target cell membranes the openings they create (MAC pores) mediate rapid osmotic lysis of the target cells. Lower, non-lytic concentrations of MACs can produce other effects. In particular, membrane insertion of small numbers of the C5b-9 complexes into endothelial cells and platelets can cause deleterious cell activation. In some cases activation may precede cell lysis.

As mentioned above, C3a and C5a are anaphylatoxins. These activated complement components can trigger mast cell degranulation, which releases histamine and other mediators of inflammation, resulting in smooth muscle contraction, increased vascular

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permeability, leukocyte activation, and other inflammatory phenomena including cellular proliferation resulting in hypercellularity. C5a also functions as a chemotactic peptide that serves to attract pro-inflammatory granulocytes to the site of complement activation.

Complement-binding recipient antibodies to donor alloantigens are considered to be the main cause of hyperacute graft rejection. Owing to pretransplant crossmatch testing, this prototype of humoral rejection is now rarely observed (Regele et al., 2001). Data are now showing that humoral immune mechanisms might contribute to other types of allograft rejection (Regele et al., 2001). High levels of panel reactive antibodies indicating humoral presensitization were found to be associated with inferior kidney graft survival (Opelz, 1992), the appearance of alloantibodies during the post-transplant period has been reported to predict poor graft outcome (Jeannet et al., 1970; Halloran et al., 1992), and selective removal of recipient IgG by immunoadsorption reversed some rejection episodes indicating the contribution of humoral immune mechanisms to rejection (Persson et al., 1995; Böhmig et al., 2000). Complement activation within a graft might indicate antibody-mediated graft injury. The complement cleavage product C4d is a marker for activation of the antibody-dependent classical pathway. Capillary C4d deposits in kidney allograft biopsies were associated with poor graft outcome.

Recently increasing evidence indicates that complement activation significantly contributes to the sensitization of allograft recipients and the development of tissue injury in allografts (Platt et al., 1999). Antibodies are the most thoroughly investigated mediators of activating the classical complement pathway. Clinically, alloantibodies are known to activate complement (Baldwin et al., 2001). Halloran and Collins indicate that C4d deposition in peritubular capillaries of renal allografts is a sensitive and diagnostic marker of acute humoral rejection that correlates strongly with the presence of circulating donor-specific antibodies (Collins et al., 1999; Halloran, 2003). Further supporting evidence is seen in animals with complement inhibition (Pratt et al., 1996; Pruitt et al., 1991; Forbes et al., 1978) or deficiency (Pratt et al., 2000; Baurer et al., 1995) which exhibit significantly reduced inflammatory injury and lowered anti-donor immune responses. In ABMR, complement is suggested to be activated by the classical pathway and to play a key role in the pathogenesis (Collard et al., 1997). Although the role of complement in HAR or acute vascular rejection (AVR) following xenotransplantation has been well documented (Platt et al., 1999), precise mechanisms of complement in the pathogenesis of ABMR following allotransplantation has not yet been elucidated.

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The C5 component of complement is cleaved to form products with multiple proinflammatory effects and thus represents an attractive target for complement inhibition within the immune-mediated inflammatory response. As described above, C5a is a powerful anaphylatoxin and chemotactic factor. Cellular activation by C5a induces the release of multiple additional inflammatory mediators (Jose et al., 1983). The complement activation pathways (classical, alternative, or mannan-binding lectin pathway) ultimately lead to the formation of the cytolytic membrane attack complex C5b-9 (Kirschfunk, 2001), which can mediate both direct tissue injury by cell lysis, and proinflammatory cell activation at sublytic doses (Saadi et al., 1995; Papadimitriou et al., 1991). Therefore, blocking both C5a and C5b-9 generation may be required for the optimal inhibition of complement-mediated inflammatory response following transplantation. At the same time, inhibition of the complement cascade at C5 does not impair the generation of C3b, preserving C3b-mediated opsonization of pathogenic microorganisms as well as solubilization and clearance of immune complexes (Liszewski, 1993).

The beneficial effect of anti-C5 mAb has previously been reported in several experimental models including myocardial reperfusion (Vakeva et al., 1998), systemic lupus erythematosus (Wang et al., 1996) and rheumatoid arthritis (Wang et al., 1995); as well as in human clinical trials (Kirschfink, 2001) of autoimmune disease, cardiopulmonary bypass and acute myocardial infarction. In addition, complement inactivation by a functionally blocking anti-C5 monoclonal Ab (mAb) prevented HAR in xenotransplantation models (Kroshus et al., 1995; Wang et al., 1999).

Methods of delaying allotransplant rejection by administration of complement inhibitors have been tested. Published PCT patent application WO 92/10205 discloses the use of a combination of cyclosporin and a soluble complement receptor (sCR1) to inhibit rejection of a cardiac allotransplant in a presensitized rat model. Complement receptor 1 binds complements C3b and C4b. Soluble forms of complement receptor 1 occur naturally or can be generated via recombinant DNA procedures. These soluble complement receptors have inhibited in vitro the consequences of complement activation (U.S. Patent 6,057,131). In WO 92/10205, rats, which had been presensitized to the cardiac allograft they were receiving, were administered cyclosporin A intramuscularly at 10 mg/kg/day beginning two days prior to transplant and continued until the time of graft rejection. Additionally, soluble complement receptor 1 (sCR1) was administered as a single intravenous bolus at 15 mg/kg immediately prior to reperfusion of the graft. Control animals with no drug treatment had the

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graft rejected at an average of 3.8 days. Those administered cyclosporin A alone rejected the grafts at an average of 57 days (this was quite variable with two rats rejecting quickly at 2 and 4 days and a third rat rejecting at 166 days). Rats administered sCR1 alone rejected the grafts at an average of 44 days. Those rats administered the combination of cyclosporin A and sCR1 rejected the grafts at an average of 147 days. The combination of chronic cyclosporin A and single bolus sCR1 was seen to result in a synergistic effect greatly prolonging the time until graft rejection. Earlier studies by Pruitt and Bollinger (1991) used a similar model of a presensitized rat allograft to show that administration of sCR1 alone to inactivate complement resulted in increased time before graft rejection.

Sims et al. (U.S. Patent 5,135,916) suggest using inhibitors of complement, e.g., CD59 or antibodies against C7 or C9 to block the formation of the C5b-9 complex, to treat the vascular endothelium of organs and tissues to be transplanted. This would prevent the C5b-9 initiated cell necrosis. The C5b-9 inactivators would be added to the perfusate or storage medium to protect the vascular lining cells from ongoing complement activation during in vitro storage. Additionally the organ or tissue would be protected from the cytolytic and thrombotic effects arising from complement activation initiated upon transplantation, thereby circumventing complement mediated acute rejection. Sims et al. (U.S. Patent 5,573,940 and U.S. Patent 6,100,443) also teach a method of expressing CD59 in the transplanted tissue or organ to protect the transplanted organ from rejection. This can be accomplished by transfecting the cells being transplanted.

Although the several drugs developed to date in combination with methods of prescreening donors and recipients to match the donor allograft to the recipient have over time increased the average length of time of survival of allografts, many allografts are nonetheless rejected during the life-time of the recipient. In general, the prior art advances have mainly been directed to overcoming acute graft rejection. Further, the role of activated terminal complement components in antibody-mediated allograft rejection has not been examined using inhibitors that specifically target the complement cascade at the C5 protein level. The methods described herein and as exemplified in the Examples advance the allotransplant art by inhibiting chronic rejection of allografts, in particular, allografts in a presensitized recipient. New methods are presented for further prolonging allograft survival by using a proper combination of immunosuppressive drugs in combination with a chronic administration of a complement inhibitor.

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Methods and Uses

The methods disclosed herein are used to prolong allograft survival. The methods generally include administering an inhibitor of complement activity in combination with one or more immunosuppressants.

Suitable complement inhibitors are known to those of skill in the art. Antibodies can be made to individual components of activated complement, e.g., antibodies to C5a, C7, C9, etc. (see, e.g., U.S. Patent 6,534,058; published U.S. patent application US 2003/0129187; and U.S. Patent 5,660,825). Proteins are known which inhibit complement-mediated lysis, including CD59, CD55, CD46 and other inhibitors of C8 and C9 (see, e.g., U.S. Patent 6,100,443). U.S. Patent 6,355,245 teaches an antibody which binds to C5 and prevents it from being cleaved into C5a and C5b thereby preventing the formation not only of C5a but also the C5b-9 complex. Proteins known as complement receptors and which bind complement are also known (see, Published PCT Patent Application WO 92/10205 and U.S. Patent 6,057,131). Use of soluble forms of complement receptors, e.g., soluble CR1, can inhibit the consequences of complement activation such as neutrophil oxidative burst, complement mediated hemolysis, and C3a and C5a production. Those of skill in the art recognize the above as some, but not all, of the known methods of inhibiting complement and its activation.

Suitable immunosuppressants include, but are not limited to, ATG or ALG, OKT3, daclizumab, basiliximab, corticosteroids, 15-deoxyspergualin, cyclosporins, tacrolimus, azathioprine, methotrexate, mycophenolate mofetil, 6-mercaptopurine, bredinin, brequinar, leflunamide, cyclophosphamide, sirolimus, anti-CD4 monoclonal antibodies, CTLA4-Ig, anti-CD154 monoclonal antibodies, anti-LFA1 monoclonal antibodies, anti-LFA-3 monoclonal antibodies, anti-CD2 monoclonal antibodies, and anti-CD45.

An allograft can include a transplanted organ, part of an organ, tissue or cell. These include, but are not limited to, heart, kidney, lung, pancreas, liver, vascular tissue, eye, cornea, lens, skin, bone marrow, muscle, connective tissue, gastrointestinal tissue, nervous tissue, bone, stem cells, islets, cartilage, hepatocytes, and hematopoietic cells.

At least part of the reason for the failure of allografts is that one response by the recipient of an allograft is the activation of complement. This results in the formation of C5a and C5b-9 which are potent proinflammatory molecules which aid in causing graft failure. Without wishing to be bound by any proposed theory, Applicants theorized that inhibiting the

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formation of C5a and C5b-9 or inhibiting C5a and C5b-9 which was present would aid in preventing graft failure. Furthermore, it was theorized that so long as the allograft is present, the recipient will continue to attempt to mount an immune response against the graft, and this response will include attempts to produce C5a and C5b-9. If not prevented, this complement response will lead to acute vascular rejection in the short term and could contribute to chronic graft rejection in the long term. Prior art methods of using inhibitors of complement activity were limited to administering these inhibitors only at the time of transplant. This was helpful in preventing acute rejection, but as the results disclosed herein illustrate, improved results are obtained by administration of such inhibitors for a longer term. This long-term administration aids in preventing a chronic rejection of the allograft as opposed to only aiding in preventing an acute rejection. The result is a longer term survival of the allograft as compared to either not administering an inhibitor of complement activity or administering such an inhibitor only at the time of transplant of the allograft. Although very commonly it is desirable that the allograft will survive for the remaining lifetime of the recipient, there are times when the allograft is needed only for a shorter length of time, e.g., a bridge organ to bridge the time until the recipient's own organ can recover on its own, at which time the allograft will no longer be needed. The length of time such a graft will be needed will vary, but will usually be longer than the time at which acute rejection would occur and may be long enough for chronic rejection to occur. This period of desired survival for a bridge graft may be several months, e.g., six months.

To prove that long-term inhibition of complement activity will prolong allograft survival, experiments were performed in which complement activation was inhibited in a chronic fashion and not merely at the time of transplant. Chronic treatment means treatment during an extended period up to the lifetime of the allograft. This can be daily treatment but is not limited to daily treatment. Chronic treatment will maintain an effective amount of the drug in the allograft recipient. For example, a preferred method is to include the anti-C5 monoclonal antibody eculizumab in the treatment. In studies of persons suffering from paroxysmal nocturnal hemoglobinuria (PNH), eculizumab has been administered at a dose of 900 mg/patient once every 12-14 days. This dosing has been found to completely and consistently block terminal complement activity and has greatly inhibited the symptoms of PNH (Hillmen et al., 2004). The administered dose is able to block the effects of complement for approximately two weeks before the eculizumab is inactivated or removed from the body. Therefore, a chronic treatment of eculizumab may be, e.g., the administration

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of 900 mg to the allograft recipient once every two weeks for the remaining life-time of the patient. Similarly, other drugs can be delivered chronically as needed, whether this is on a daily basis or another schedule is required to maintain an effective amount of the drug in the allograft recipient. Because it is well known that graft rejection can be caused by more than just complement activation, e.g., by T cell activity, the experiments included immunosuppressants such as cyclosporin to further aid in preventing graft rejection.

A preferred method of inhibiting complement activity is to use a monoclonal antibody which binds to complement C5 and prevents C5 from being cleaved. This prevents the formation of both C5a and C5b-9 while at the same time allowing the formation of C3a and C3b which are beneficial to the recipient. Such antibodies that are specific to human complement are known (U.S. Patent 6,355,245). These antibodies disclosed in U.S. Patent 6,355,245 include both a whole or full-length antibody (now named eculizumab) and a single-chain antibody (now named pexelizumab). A similar antibody against mouse C5 is called BB5.1 (Frei et al., 1987). BB5.1 was utilized in the experiments set forth below. Antibodies to inhibit complement activity need not be monoclonal antibodies. They can be, e.g., polyclonal antibodies. They may additionally be antibody fragments. An antibody fragment includes, but is not limited to, an Fab, F(ab'), $F(ab')_2$, a single-chain antibody, a domain antibody and an Fv. Furthermore, it is well known by those of skill in the art that antibodies can be humanized (Jones et al., 1986), chimerized, or deimmunized. An antibody may also comprise a mutated Fc portion, such that the mutant Fc does not activate complement. The antibodies to be used in the present disclosure may be any of these. It is preferable to use humanized antibodies when the recipient of the allograft is a human.

Administration and Formulations

Administration of the inhibitor of complement activity is performed according to methods known to those of skill in the art. These inhibitors are administered preferably before the time of allograft transplantation or at the time of transplantation with administration continuing in a chronic fashion. These inhibitors can additionally be administered during a rejection episode in the event such an episode does occur.

The present disclosure also provides uses of a drug that inhibits complement activity and an immunosuppressive agent in the manufacture of a medicament or medicament package. Such medicament or medicament package is useful in prolonging allograft survival in a recipient, in particular, chronic survival of the allograft. In preferred embodiments, the

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medicament or medicament package is formulated and prepared such that it is suitable for chronic administration to the recipient of the allograft, for example, stable formulations are employed. In certain embodiments, the medicament or medicament package is formulated and prepared such that it is suitable for concurrent administration of the drug that inhibits complement activity and the immunosuppressive drug to the recipient of the allograft. In certain embodiments, the medicament or medicament package is formulated and prepared such that it is suitable for sequential (in either order) administration of the drug that inhibits complement activity and the immunosuppressive drug to the recipient of the allograft.

A pharmaceutical package of the present disclosure may comprise a drug that inhibits complement activity and at least one immunosuppressive agent. The pharmaceutical package may further comprise a label for chronic administration. The pharmaceutical package may also comprise a label for self-administration by a patient, for example, a recipient of a transplant graft, or instructions for a caretaker of a recipient of a transplant graft. In certain embodiments, the drug and the agent in the pharmaceutical package are in a formulation or separate formulations that are suitable for chronic administration and/or self-administration.

The present disclosure also provides lyophilized formulations and formulations suitable for injection. Certain embodiments provide a lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant. In preferred embodiments, the antibody formulation is suitable for chronic administration, for example, the antibody formulation stable. Alternative embodiments provide an injection system comprising a syringe; the syringe comprises a cartridge containing an antibody that inhibits complement activity and is in a formulation suitable for injection.

An antibody employed in various embodiments of the present disclosure preferably inhibits the formation of terminal complement or C5a. In certain embodiments, antibody inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment. The whole antibody or antibody fragment may be a human, humanized, chimerized or deimmunized antibody or antibody fragment. In certain embodiments, the whole antibody or antibody fragment may inhibit cleavage of complement C5. In certain embodiments, the antibody fragment is a Fab, an F(ab')2, an Fv, a domain antibody, or a single-chain antibody. In preferred embodiments, the antibody fragment is pexelizumab. In alternative preferred embodiments, the whole antibody is eculizumab.

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In certain embodiments, a drug, such as an antibody, that inhibits complement activity is present in unit dosage form, which can be particularly suitable for self-administration. Similarly, an immunosuppressive agent of the present disclosure may also be present in unit dosage form. A formulated product of the present disclosure can be included within a container, typically, for example, a vial, cartridge, prefilled syringe or disposable pen. A doser such as the doser device described in U.S. Patent 6,302,855 may also be used, for example, with an injection system of the present disclosure.

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

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

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

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

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

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

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

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

Accordingly, a stable lyophilized antibody formulation can be prepared using a lyoprotectant (preferably a sugar such as sucrose or trehalose), which lyophilized formulation

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can be reconstituted to generate a stable reconstituted formulation having an antibody concentration which is significantly higher (e.g. from about 2-40 times higher, preferably 3-10 times higher and most preferably 3-6 times higher) than the antibody concentration in the pre-lyophilized formulation. Such high protein concentrations in the reconstituted formulation are considered to be particularly useful where the formulation is intended for subcutaneous administration. Despite the very high protein concentration in the reconstituted formulation, the reconstituted formulation can be stable (i.e. fails to display significant or unacceptable levels of chemical or physical instability of the protein) at 2-8 °C. for at least about 30 days. See U.S. Patent 6,821,515. In certain embodiments, the reconstituted formulation is isotonic.

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

The present disclosure also provides a method for preparing a formulation comprising the steps of: (a) lyophilizing a mixture of an antibody and a lyoprotectant; and (b) reconstituting the lyophilized mixture of step (a) in a diluent such that the reconstituted formulation is isotonic and stable.

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

An injection system of the present disclosure may employ a medication delivery pen as described in U.S. Patent 5,308,341. Medication delivery pens have been developed to facilitate the self-administration of medication. A medication of the present disclosure can be a drug that inhibits complement activity, for example an antibody specific to complement C5,

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and/or an immunosuppressive agent. One medication delivery pen includes a vial holder into which a vial of insulin or other medication may be received. The vial holder is an elongate generally tubular structure with proximal and distal ends. The distal end of the vial holder includes mounting means for engaging a double-ended needle cannula. The proximal end also includes mounting means for engaging a pen body which includes a driver and dose setting apparatus. A disposable medication containing vial for use with the prior art vial holder includes a distal end having a pierceable elastomeric septum that can be pierced by one end of a double-ended needle cannula. The proximal end of this vial includes a stopper slidably disposed in fluid tight engagement with the cylindrical wall of the vial. This medication delivery pen is used by inserting the vial of medication into the vial holder. A pen body then is connected to the proximal end of the vial holder. The pen body includes a dose setting apparatus for designating a dose of medication to be delivery by the pen and a driving apparatus for urging the stopper of the vial distally for a distance corresponding to the selected dose.

The user of the pen mounts a double-ended needle cannula to the distal end of the vial holder such that the proximal point of the needle cannula pierces the septum on the vial. The patient then selects a dose and operates the pen to urge the stopper distally to deliver the selected dose. The dose selecting apparatus returns to zero upon injection of the selected dose. The patient then removes and discards the needle cannula, and keeps the prior art medication delivery pen in a convenient location for the next required medication administration. The medication in the vial will become exhausted after several such administrations of medication. The patient then separates the vial holder from the pen body. The empty vial may then be removed and discarded. A new vial can be inserted into the vial holder, and the vial holder and pen body can be reassembled and used as explained above.

Accordingly, a medication delivery pen generally has a drive mechanism for accurate dosing and ease of use. A dosage mechanism such as a rotatable knob allows the user to accurately adjust the amount of medication that will be injected by the pen from a prepackaged vial of medication. To inject the dose of medication, the user inserts the needle under the skin and depresses the knob once as far as it will depress. The pen may be an entirely mechanical device or it may be combined with electronic circuitry to accurately set and/or indicate the dosage of medication that is injected into the user. See U.S. Patent 6,192,891.

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The present disclosure also presents controlled-release or extended-release formulations suitable for chronic and/or self-administration of a medication.

The various formulations can be administered to a patient in need of treatment (e.g., a recipient of an allograft) with the medication (e.g., an antibody of the present disclosure and at least one immunosuppressive agent) by intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

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

The present methods and uses are described with reference to the following Examples, which are offered by way of illustration and are not intended to limit the disclosure in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized. The following abbreviations are used herein: ABMR, antibody-mediated rejection; ACHR, accelerated humoral rejection; ACR, acute cellular rejection; AVR, acute vascular rejection; CsA, cyclosporin; CyP, cyclophosphamide; HAR, hyperacute rejection; MCP-1, monocyte chemotactic protein 1; MST, mean survival time; POD, postoperative day.

Example 1

Methods

Animals and Immunosuppressive Drugs Male adult C3H (H-2^k) mice and BALB/c (H-2^d) mice (Jackson Labs, Bar Harbor, Maine) weighing 25-30 g were chosen as donors and recipients, respectively. In the groups receiving immunosuppression, the recipients were injected with CsA (15 mg/kg/day, s.c., daily from day 0 to endpoint rejection or until day 100), or with CyP (40 mg/kg/day, i.v., on day 0 and 1), or with anti-C5 mAb (clone BB5.1, Alexion Pharmaceuticals Inc., 40 mg/kg/day, i.p., day 0–2, followed by twice a week, day 0-60). Animals were housed under conventional conditions at the Animal Care Facility,

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University of Western Ontario, and were cared for in accordance with the guidelines established by the Canadian Council on Animal Care. Olfert et al., 1993.

Skin Presensitization Full-thickness skin grafts taken from C3H donors were cut into square pieces of 1×1 cm^2 and transplanted onto the back of the BALB/c recipients' thorax one week prior to heart transplantation from the same donors. Rejection was defined as complete necrosis of the skin grafts.

Abdominal and Cervical Cardiac Transplantation Seven days after skin presensitization, C3H mouse hearts were transplanted into the abdomen of presensitized BALB/c recipients by anastomosing the donor aorta and recipient aorta, and the donor pulmonary artery and recipient inferior vena cava. In the groups with re-transplantation, second heart grafts harvested from either naive C3H mice or long-term surviving presensitized BALB/c recipients were transplanted into the cervical area of the recipients carrying a long-term surviving first abdominal heart graft by anastomosing the donor aorta and recipient carotid artery, and the donor pulmonary artery and recipient external jugular vein (end-to-side). The heart grafts were monitored daily until rejection unless otherwise indicated and rejection was defined as complete cessation of pulsation.

Experimental Groups Presensitized recipients were randomly assigned to eight groups, each consisting of eight animals: Group 1, mice with no treatment; Group 2, mice treated with CsA; Group 3, mice treated with CyP; Group 4, mice treated with CsA plus CyP; Group 5, mice treated with anti-C5 mAb; Group 6, mice treated with anti-C5 mAb plus CsA; Group 7, mice treated with anti-C5 mAb plus CyP; Group 8, mice treated with anti-C5 mAb in combination of CsA and CyP. When cardiac impulses were no longer palpable or at POD100, the grafts were removed for routine histology, immunohistochemistry and western blot analysis, serum samples were collected for flow cytometric analysis and complement hemolytic assay. Five additional animals were placed and sacrificed in groups 6 and 8 on POD3 (MST for groups 1-5, 7) to allow for comparisons at a uniform time point. Serum samples were also collected on POD 11, 21, 28 and 60 in Group 8 for detecting the sequential changes of anti-donor antibody levels and complement activity. In addition, when triple therapy treated presensitized recipients carried a first heart graft for 100 days, they were re-transplanted with a second heart. A naive C3H heart or a 100-day surviving C3H heart from another presensitized BALB/c recipient was used as the second heart. Eight animals were included in each re-transplant group.

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Graft Histology Tissue samples were fixed in 10% buffered formaldehyde. Specimens were then embedded in paraffin, and sectioned for H&E staining. The microscopic sections were examined in a blinded fashion for severity of rejection by a pathologist. Criteria for graft rejection included the presence of vasculitis, thrombosis, hemorrhage and lymphocyte infiltration. These changes were scored as: 0, no change; 1, minimum change; 2, mild change; 3, moderate change; or 4, marked change.

Immunohistochemistry Four micrometer sections were cut from tissue samples embedded in Tissue-Tek O.C.T gel (Optimum Cutting Temperature, Skura Finetek, Torrance, CA) mounted on gelatin-coated glass microscope slides and stained by a standard indirect avidin-biotin immunoperoxidase staining method using an Elite Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA). Specimens were stained for $CD4^+$ and $CD8^+$ cells with biotin-conjugated rat anti-mouse CD4 mAb (clone YTS 191.1.2, Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) and biotin-conjugated rat anti-mouse CD8 mAb (clone 53-6.7, Pharmingen, Franklin Lakes, NJ), respectively. Intragraft monocyte/macrophage infiltration was detected by staining with biotin-conjugated rat anti-mouse Mac-1 mAb (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada). Mouse IgG and IgM deposition in grafts was detected using biotin-conjugated goat anti-mouse-IgG and goat anti-mouse-IgM (Cedarlane). For identification of complement deposition, sections were serially incubated with goat anti-C3 or anti-C5 polyclonal Abs (Quidel, San Diego, CA), biotinylated rabbit anti-goat IgG (Vector Laboratories), and HRP-conjugated-streptavidin (Zymed Laboratories, South San Francisco, CA). Slides were washed with phosphate-buffered saline between steps, and examined under light microscopy. Negative controls were performed by omitting the primary antibodies. The immunostaining was scored in five high-power fields of each section, and five independent experiments were performed. The sections of immunoperoxidase staining were graded from 0 to 4+ according to the staining intensity: 0, negative; 1+, equivocal; 2+, weak staining; 3+, moderate staining; and 4+, very intensive staining.

Flow Cytometry The circulating anti-donor specific IgG and IgM antibodies were evaluated in the recipient serum by FACScan flow cytometry (Becton Dickinson, Mountain View, CA). Glotz et al., (1993); Tyan et al. (1994). Briefly, C3H mouse splenocytes were isolated and incubated at 37 °C for 30 minutes with serum from naive control and experimental groups. To stain for total IgG, IgG1, IgG2a, IgG2b and IgM, the cells were washed and incubated with FITC-conjugated goat antibody specific for the Fc portion of mouse IgG or with

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phycoerythrin-conjugated goat antibody specific for the μ-chain of mouse IgM (Jackson ImmunoResearch Laboratories, West Grove, PA), or with FITC-conjugated goat anti-mouse IgG1 (CALTAG Laboratories, Burlingame, CA), or with FITC-conjugated goat anti-mouse IgG2a (CALTAG), or with FITC-conjugated goat anti-mouse IgG2b (CALTAG). After 1 hour of staining at 4 °C, the cells were washed with PBS, resuspended at 5×10^6/mL, and analyzed by flow cytometry for mean channel fluorescence intensity, which represents the antibody-binding reactivity.

Complement Hemolytic Assay The purified anti-C5 mAb was serially diluted twofold (175-0.1 μg/ml) in GVB^{2+} buffer (gelatin Veronal-buffered saline: 0.1% gelatin, 141 mM NaCl, 0.5 mM $MgCl_2$, 0.15 mM $CaCl_2$, and 1.8 mM sodium barbital) and added in triplicate (50 μl/well) to a 96-well plate. BALB/c mouse serum was diluted to 40% v/v with GVB^{2+} buffer and added (50 μl/ml) to the rows of the same 96-well plate such that the final concentration of BALB/c mouse serum in each well was 20%. The plate was then incubated at room temperature for approximately 30 min while chicken erythrocytes were prepared. Chicken erythrocytes were washed 5×1 ml with GVB^{2+} buffer and resuspended to a final concentration of 5×10^7/ml in GVB^{2+}. Four milliliters of the chicken erythrocytes were sensitized by adding anti-chicken RBC polyclonal antibody (Intercell Technologies, Hopewell, NJ, 0.1% v/v) and the cells were incubated at 4 °C for 15 min with frequent vortexing. The cells were then washed 2×1 ml with GVB^{2+} and resuspended to a final volume of 2.4 ml in GVB^{2+}. The chicken erythrocytes (30 μl/well, 2.5×10^6 cells) were added to the plate containing serum and anti-C5 mAb as described above, mixed well, and incubated at 37 °C for 30 min. The plate was then centrifuged at 1000×g for 2 min, and 85 μl of the supernatant was transferred to a new 96-well microtiter plate. The plate was read at OD 415 nm using a microplate reader and the percentage of hemolysis was determined using this formula:

$$\%\ \text{hemolysis} = 100 \times \frac{(\text{OD sample}) - (\text{OD } GVB^{2+} \text{ control})}{(\text{OD 100\% lysed control}) - (\text{OD } GVB^{2+} \text{ control})}$$

with 100% lysed control obtained by the addition of 100 μl GVB^{2+} containing 0.1% NP-40 to the 30 μg/ml of chicken erythrocytes as prepared above.

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Western Blot Analysis Sonication of frozen heart samples was performed in RIPA lysis buffer (Santa Cruz Biotechnology, Inc.) at 4 °C for 1 minute at 10-second intervals, followed by microcentrifugation at 13,000 rpm for 10 minutes at 4 °C. Clarified supernatants were immediately quantitated in triplicate for protein content using Detergent-compatible protein assay kit (BIO-RAD). Heart lysates (10μg protein/well) were separated on NuPAGE, 4-12% gradient Bis-Tris gels and MES buffer system (Invitrogen) and transferred to polyvinylidene difluoride (PVDF) membrane (0.45 μm pore size; Invitrogen) using a semi-dry transfer apparatus (BIO-RAD). Membranes were cut appropriately at the correct molecular weights to allow the development of the blots with two different primary antibodies per blot such that each blot was exposed to a test antibody and an internal control antibody to insure equal sample loading. The test primary antibodies including anti-Bcl-2 (N-19) rabbit polyclonal sera (Santa Cruz Biotechnology, Inc.) and anti-Bcl-XS/L (M-125) rabbit polyclonal sera (Santa Cruz Biotechnology, Inc.) were used to detect intragraft expression of Bcl-2 and Bcl-xl proteins. Anti-calsequestrin rabbit polyclonal sera (Calbiochem) were used as internal control primary antibody (Kobayashi et al., 1999). Detection of primary antibody binding was performed as previously described (Arp et al., 1996) by exposing washed incubated blots to a polyclonal goat anti-rabbit IgG fraction conjugated to horseradish peroxidase (HRP; Roche Laboratories) and then appropriately developed by exposure to enhance chemiluminescence for HRP-conjugated antibodies (Roche Laboratories).

Statistical Analysis The data were reported as the mean ± SD. Allograft survival among experimental groups was compared using the rank-log test. Histological and immunohistological findings were analyzed using the Mann-Whitney U test. Flow cytometric data and western blot data were analyzed using one-way ANOVA. Differences with p values less than 0.05 were considered significant.

Example 2

Presensitization with C3H donor skin graft induces antibody-mediated ACHR in heart allografts of BALB/c recipients.

To develop a suitable small animal model that mimics presensitized patients in the clinic and to study ABMR, a novel, fully MHC-mismatched mouse ABMR model has been developed through presensitization of mouse recipients. In this model, BALB/c recipients were presensitized with C3H donor skin grafts one week prior to heart transplantation from the same donor. Seven days after donor skin presensitization, serum level of anti-donor IgG,

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but not IgM antibody was markedly elevated and reached to a peak level in the presensitized BALB/c recipients (Figure 1A). Heart transplantation from same donor was then performed in these highly sensitized recipients. Without immunosuppression, C3H heart grafts were rapidly rejected in 3.1 ± 0.4 days by ACHR, characterized by severe thrombosis, hemorrhage and infarction (Figure 1B-a). In contrast, same heart grafts in unsensitized BALB/c recipients (with mean survival time, MST of 8.2 ± 0.8 days) show the normal histology on post-operative day (POD) 3 (Figure 1B-b). When compared to unsensitized BALB/c recipients at the same day, heart grafts in presensitized animals revealed massive IgG antibody and complement (C3 and C5) deposition, but minimal $CD4^+$ and $CD8^+$ cell infiltration (Table 1). Furthermore, circulating anti-donor IgG levels in presensitized recipients were significantly higher than those of unsensitized same recipients receiving a heart graft on POD3 ($P< 0.01$, Figure 1C). However, anti-donor IgM remained at very low levels both in circulation (Figure 1C) and in heart grafts (Table 1) and it showed no significant difference between unsensitized and presensitized recipients. In addition, normal levels of complement hemolytic activity were shown in both presensitized and unsensitized heart recipients without treatment (Figure 1D). These data indicate that this is an ideal transplant model to study ABMR in presensitized recipients in which complement plays an important role in the pathogenesis.

Table 1.

Comparison of immunohistological changes of C3H heart allografts in unsensitized and presensitized BALB/c recipients on POD3

Groups	Unsensitized	Presensitized
IgG	1+	4+
IgM	1+	1+
C3	2+	3+
C5	2+	3+
CD4	0	1+
CD8	0	1+

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Grades for immunoperoxidase staining: 0, negative; 1+, equivocal; 2+, weak; 3+, moderate; 4+, intense.

Anti-C5 mAb in combination with CsA and CyP prevents ABMR and achieves indefinite heart allograft survival in presensitized mouse recipients.

Complement has been shown to play an important role in ABMR. However, the inhibitory effect of functionally blocking terminal complement cascade at the C5 level in highly sensitized recipients is unknown. In the study presented herein, the presensitized model was used to study the efficacy of anti-C5 mAb either alone or combined with CsA and /or CyP in prevention of ABMR. As presented in Figure 2A, treatment with either CsA or CyP or the two drugs in combination did not prevent ABMR and grafts were rejected in 3.0 ± 0.0 days, 3.3 ± 0.5 days and 3.5 ± 0.6 days, respectively with typical pathological features of ACHR including intravascular thrombosis and interstitial hemorrhage (Figure 2B-b, c, d), which were indistinguishable from heart grafts in untreated presensitized BALB/c recipients (Figure 2B-a). Anti-C5 monotherapy or combined with CyP was not able to improve graft survival and heart grafts were rejected by ACHR (Figure 2B-e, f) in 3.5 ± 0.6 days and 3.2 ± 0.4 days, respectively (Figure 2A). Although the combination therapy of anti-C5 mAb and CsA, the protocol capable of inducing long-term heart allograft survival in unsensitized animals, marginally prolonged graft survival in this presensitized model, heart grafts were also rejected by severe humoral rejection with vasculitis, thrombosis, hemorrhage and minimal cell infiltration (Figure 2B-g) in 11.9 ± 1.8 days (Figure 2A). In contrast, triple therapy of anti-C5 mAb in combination of CsA and CyP achieved indefinite heart graft survival over 100 days (Figure 2A) in presensitized animals ($P<0.01$ vs. the animals without treatment or treated with either monotherapy or two drugs in combination) with no evidence of rejection (Figure 2B-h). In this presensitized mouse model, as shown in Table 2, only minor intragraft $CD4^+$ and $CD8^+$ cell infiltration was observed in the recipients that rejected their heart grafts within 3 days. However, the number of these T cells was slightly increased if heart grafts survived longer in anti-C5 mAb plus CsA-treated recipients at the time of rejection (POD11) and in triple therapy-treated recipients at early stages of graft survival (e.g. POD11). Furthermore, with continuous treatment of CsA in the triple therapy group, $CD4^+$ and $CD8^+$ cell infiltration was inhibited in long-term surviving heart grafts on POD60 and 100. In addition, moderate intragraft Mac-1^+ cell infiltration, including monocytes and macrophages, was found in untreated and CsA-, CyP- or CsA plus CyP-treated animals,

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while the infiltration of these cells was significantly reduced in anti-C5 mAb treated animals (Table 2). These results indicate that functionally blocking anti-C5 mAb enables the use and efficacy of conventional immunosuppressive agents, thereby preventing ABMR and achieving indefinite heart graft survival in presensitized recipients.

Table 2.

Grades for immunoperoxidase staining of heart allografts

in presensitized mouse recipients at necropsy

Groups	Date for sample collection (POD)	C3	C5	**CD4**	CD8	Mac-1	IgG	IgM
Untreated	3	3+	3+	1+	1+	3+	4+	1+
CsA	3	3+	3+	1+	1+	3+	4+	1+
CyP	3	3+	3+	1+	1+	3+	3+	1+
CsA+CyP	3	3+	3+	1+	1+	3+	3+	1+
Anti-C5mAb	3	3+	0	1+	1+	2+	4+	1+
Anti-C5mAb+CsA	11	3+	0	2+	2+	2+	4+	1+
Anti-C5mAb+CyP	3	3+	0	1+	1+	2+	3+	1+
Anti-C5mAb+CsA+CyP	3	3+	0	1+	1+	2+	3+	1+
Anti-C5mAb+CsA+CyP	11	3+	0	2+	2+	1+	3+	1+
Anti-C5mAb+CsA+CyP	60	3+	0	1+	1+	0	2+	1+
Anti-C5mAb+CsA+CyP	100	3+	2+	0	0	0	2+	1+

Grades: 0, negative; 1+, equivocal; 2+, weak; 3+, moderate; 4+, intense.

Anti-C5 mAb completely inhibits total complement hemolytic activity and local C5 deposition in presensitized recipients receiving a heart allograft.

Anti-C5 mAb was previously shown to block the cleavage of complement protein C5 into the proinflammatory molecules C5a and C5b-9 (Kroshus et al., 1995), and to completely and consistently block terminal complement activity in mice (Wang et al., 1999). In the current study, terminal complement activity was measured by assessing the ability of

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recipient mouse sera to lyse antibody presensitized chicken erythrocytes and was compared at the same time-point (POD3). Treatment of mice with either CsA or CyP or the two drugs in combination had no effect on terminal complement activity, while treatment with anti-C5 mAb either alone or combined with CsA or/and CyP completely inhibited this activity (Figure 3; $P<0.01$, vs. naive and untreated animals, as well as CsA-, CyP-, or CsA plus CyP-treated animals). In addition, sera obtained from anti-C5 mAb treated animals at several earlier time points showed similarly diminished hemolytic activity, suggesting that serum terminal complement was inhibited throughout the treatment period. Furthermore, local C5 deposition in heart grafts was completely prevented in the anti-C5 mAb treated presensitized recipients, but not in untreated, or CsA-, CyP- and CsA plus CyP-treated presensitized animals (Table 2). As predicted, treatment with anti-C5 mAb did not prevent C3 deposition in the grafts (Table 2). These results suggest anti-C5 therapy completely blocks total complement activity after cardiac allografting in highly sensitized recipients.

Long-term surviving heart grafts in presensitized animals are resistant to humoral injury in the presence of low level of anti-donor antibodies and complement- a situation of accommodation.

To further investigate the role of anti-C5 mAb in humoral rejection, anti-donor alloantibody levels were measured in recipient sera by flow cytometry and intragraft antibody deposition by using immunostaining techniques in different groups. Figure 4A shows that on POD3 untreated presensitized BALB/c recipients had high levels of circulating anti-donor IgG antibodies. When presensitized recipients receiving either monotherapy or two drugs in combination, CsA and/or CyP partially down-regulated circulating anti-donor IgG levels, while treatment with anti-C5 mAb either alone or combined with CsA or CyP did not further affect anti-donor antibody levels at the same day. In contrast, with triple therapy of anti-C5 mAb, CsA and CyP, a high level of circulating anti-donor IgG was gradually downregulated and reached a low level on POD60, thereafter remaining at this level until day 100 (Figure 4B). Similar to levels of circulating antibodies in the different treatment groups, Table 2 shows that strong deposition of anti-mouse IgG was present in the rapidly rejected heart grafts of presensitized animals with no treatment or treated with monotherapy or two drugs in combination therapy. Interestingly, with triple therapy, IgG antibody deposition was gradually attenuated to a mild level in the long-term surviving heart grafts on POD 100 (Figure 4C-a, Table 2). In this model, IgM remained at very low levels in either circulation (Figure 4A, B) or transplanted heart grafts (Figure 4C-b, Table 2) in presensitized recipients

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with or without treatment. In addition, treatment with anti-C5 mAb eliminated complement activity to an undetectable level until day 60, followed by a progressive recovery to predepletion levels on POD 100 after discontinuation of anti-C5 therapy in presensitized mouse recipients receiving triple therapy (Figure 4D). Furthermore, intragraft C5 deposition was also detected in 100-day surviving presensitized animals (Table 2). These data demonstrate that ongoing transplant accommodation occurs in triple therapy treated presensitized recipients despite the presence of anti-graft antibodies and complement activation.

Anti-C5 mAb in combination with CsA and CyP reduces the IgG1/IgG2a ratio and leads to a shift in IgG subclass to IgG2b in recipients with accommodated grafts.

To determine whether anti-C5 mAb-based triple therapy would induce a shift in IgG subclass, which may be associated with accommodation, serum levels of anti-donor IgG subclasses of IgG1, IgG2a and IgG2b were compared between untreated recipients and the recipients with accommodated heart graft. Sera from untreated recipients contained predominant IgG1 isotype, indicated by a high ratio of IgG1/IgG2a (Figure 5A). In contrast, a significant reduction in the ratio of IgG1/IgG2a was observed in the recipients carrying accommodated grafts (Figure 5A, $P<0.01$). Furthermore, presensitized recipients with the accommodated heart grafts displayed an increased level of anti-donor IgG2b as compared to the same recipients with rejected grafts (Figure 5B, $P<0.01$). In addition, the pattern of IgG isotypes in the recipients treated with either monotherapy or two drugs in combination is indistinguishable from that of untreated animals. These data indicate that anti-donor IgG1 isotype may be associated with graft rejection, while production of anti-donor IgG2b subclass may function as a protective antibody and plays an important role in the induction of accommodation.

Anti-C5 mAb in combination with CsA and CyP induces intragraft Bcl-2 and Bcl-xl expression in highly sensitized mouse recipients.

To determine whether a causal relationship exists between intragraft expression of protective proteins and graft resistance to humoral injury in this model, western blot analysis was employed to detect proteins of interest in heart graft tissues from highly sensitized mouse recipients. Long-term surviving heart grafts were found to express high levels of Bcl-2 and Bcl-xl proteins on POD100, and these proteins were detected as early as 12 days after heart transplantation in highly sensitized recipients receiving anti-C5 mAb-based triple therapy

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(Figure 6). In contrast, there were no Bcl-2 and Bcl-xl proteins expressed on heart grafts of untreated animals (Figure 6) or animals treated with either monotherapy or two drugs in combination therapy. This result suggests that graft resistance to humoral injury in indefinite surviving animals is associated with the protection provided by Bcl-2 and Bcl-xl proteins in this presensitized model.

Presensitized recipients with an accommodating first heart graft accept a second accommodated heart graft but reject a second naive heart graft from the same donors.

The ability of accommodated grafts to resist rejection has not been tested directly under pathophysiological conditions where naive grafts undergo rejection following allotransplantation. In this model, to determine whether presensitized recipients with an accommodating first heart graft will accept a second accommodated graft but reject a second naive graft, we performed re-transplantation scenarios. After the accommodated C3H heart grafts have survived to the 100-day point, the time at which low levels of alloantibodies were detected (Figure 4B) and complement activity has returned to pretreatment levels (Figure 4D), in presensitized BALB/c recipient treated with anti-C5 mAb-based triple therapy, these recipients received a second heart graft. Specifically, either a naive (Figure 7A) or a 100-day accommodated C3H heart (Figure 7B) from another presensitized BALB/c recipient was transplanted into the neck of the presensitized recipients carrying an accommodating first C3H heart. These recipients rejected a second naive heart at 6.6 ± 1.1 days (Figure 8A) with severe AVR (Figure 8B-a) while the first heart continued to survive. In contrast, when the accommodated hearts that had been already surviving for 100 days in different presensitized mice were used as second grafts, these grafts were accepted by the presensitized recipients carrying an accommodating first heart graft (Figure 8A). There was no sign of rejection in those accommodated second heart grafts 90 days after second transplantation (Figure 8B-b). These data indicate that accommodated grafts become resistant to the effects of anti-donor antibodies and complement that normally mediate allograft rejection in these presensitized recipients. Furthermore, the fact that the host of the accommodated graft rejected a new graft suggests that accommodation involves changes to the graft.

Presensitized recipients being treated with CsA reject accommodated heart grafts.

Another re-transplantation was performed to determine whether accommodation in this presensitized model would be caused by the changes in the grafts and/or the recipients. Specifically, after C3H heart grafts have been accommodated in presensitized BALB/c mice

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for 100 days, the accommodated heart graft will then be re-transplanted into a second presensitized BALB/c recipient being treated with CsA alone (Figure 7C), a therapy that can prevent cellular rejection but cannot prevent accelerated humoral rejection of a fresh C3H heart in presensitized recipients. The accommodated C3H heart grafts were rapidly rejected in CsA treated presensitized BALB/c recipients. After re-transplantation, the pathology in accommodated heart grafts was changed from normal (Figure 9A) to severe ACHR with massive interstitial hemorrhage but few cell infiltrates (Figure 9B). In addition, high levels of anti-donor IgG and normal levels of complement hemolytic activity in these recipients receiving an accommodated C3H heart were similar to those of CsA treated presensitized recipients receiving a naive C3H heart. This result further indicates that accommodation induced by anti-C5 mAb-based triple therapy can originate from mechanisms involving changes not only to the graft, but also to the recipient.

Example 3

Acute Vascular Rejection in a Heart Transplantation Model

Experiments were performed to determine whether inclusion of an inhibitor of formation of terminal complement would attenuate acute vascular rejection and whether the use of such an inhibitor in conjunction with an immunosuppressant would achieve long-term allograft survival. In this set of experiments an anti-C5 monoclonal antibody was used in conjunction with cyclosporin. The model used was an allograft heterotopic heart transplant from C3H mice into BALB/c mice. This model is a stringent acute vascular rejection model with the C3H and BALB/c mice being strongly MHC mismatched. The transplantations and other methods were performed as described in Wang et al. (2003).

Heterotopic Cardiac Transplantation

Intra-abdominal heterotopic cardiac transplantation was performed as previously described by Wang et al. (2003). Briefly, a median sternotomy was performed on the donor, and the heart graft was slowly perfused in situ with 1.0 ml of cold heparinized Ringer's lactate solution through the inferior vena cava and aorta before the superior vena cava and pulmonary veins were ligated and divided. The ascending aorta and pulmonary artery were transected, and the graft was removed from the donor. The graft was then revascularized with end-to-side anastomoses between the donor's pulmonary artery and the recipient's inferior vena cava as well as the donor's aorta and the recipient's abdominal aorta using 11-0 nylon suture. The beating of the grafted heart was monitored daily by direct abdominal palpation.

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The degree of pulsation was scored as: A, beating strongly; B, noticeable decline in the intensity of pulsation; or C, complete cessation of cardiac impulses. When cardiac impulses were no longer palpable, the graft was removed for routine histology. In certain instances, mice in which the graft was still functioning were sacrificed to perform histology.

Results

Mice (male 8-12 week old mice weighing 25 – 30 g) were split into six experimental groups with six to eight mice per group. Transplant occurred on day 0. Histological changes were checked at the endpoint (the endpoint being graft failure) or in some cases a mouse was sacrificed prior to graft failure. The dosage of BB5.1 which was administered (40 mg/kg body weight three times per week) was known from prior studies to completely inhibit terminal complement activity.

Group 1' (control) - mice were administered 0.75 mL of saline intraperitoneally on days -1, 0, 1 and 2. Subsequently these mice were treated with 0.75 mL of saline intraperitoneally three times per week (Monday, Wednesday, Friday) until the endpoint.

Group 2' (cyclosporin A alone) - mice were administered 15 mg/kg body weight of cyclosporin A subcutaneously on a daily basis beginning at day 0 (day of transplant) until the endpoint.

Group 3' (anti-complement antibody alone) - mice were administered the anti-mouse C5 antibody BB5.1 (Frei et al., 1987) at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until the endpoint.

Group 4' (anti-complement antibody until day 14 post-transplant plus cyclosporin A) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intravenously on days -1 through day 14 and were also administered cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until the endpoint. Note that this differs from the other groups in that the BB5.1 was administered intravenously and on a daily basis.

Group 5' (anti-complement antibody until day 28 post-transplant plus cyclosporin A) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until day 28 and were also administered

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cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until the endpoint.

Group 6' (anti-complement antibody chronically until 100 days plus cyclosporin) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until 100 days and were also administered cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until 100 days.

The results of this experiment are shown in Tables 3 and 4. Table 3 shows the survival time for the grafts. Table 4 sets forth the histological scores.

Table 3

Allograft Survival

Group (Treatment)	Individual Survival (days)	Mean Survival Time (days)
1'. Saline	8, 8, 8, 8, 8, 9	8.3 ± 0.5
2'. Cyclosporin A	14, 15, 15, 16, 16, 16, 17	15.5 ± 1.1
3'. BB5.1	7, 8, 8, 8, 8, 9	8.0 ± 0.6
4'. BB5.1 until day 14 + cyclosporin A	35, 38, 43, 45, 46, 47	42.3 ± 4.8
5'. BB5.1 until day 28 + cyclosporin A	77, 80, 80, 81, 82	80 ±1.9
6'. BB5.1 until day 100 + cyclosporin A	>100 days (7 mice; one sacrificed for histology)	> 100 days

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Table 4

Median Scores of Histological Changes of Heart Allografts at Necropsy

Groups	Vasc*	Infarc	Lymph	Throm	Hemo	Fibrin	PMN
1'. Saline (endpoint)	3.0	3.0	1.0	4.0	3.0	3.0	3.0
2'. Cyclosporin A (endpoint)	2.0	1.0	2.0	3.0	2.0	2.0	3.0
3'. BB5.1 (endpoint)	2.0	1.0	2.0	2.0	1.0	0.0	0.0
4'. BB5.1 until day 14 + cyclosporin A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
5'. BB5.1 28 days + Cyclosporin A (post-operative day 8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5'. BB5.1 28 days + Cyclosporin A (endpoint)	0.0	0.0	1.0	1.0	2.0	1.0	0.0
6'. BB5.1 until day 100 + Cyclosporin A (post-operative day 100)	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Median scores: 0 - normal; 1- minimum change; 2 - mild change; 3 - moderate change; 4 - marked change. N/A - not available.

*Vasc - vasculitis; Infar - infarction; Lymph - lymphocyte infiltration; Throm - thrombosis; Hemo - hemorrhage; Fibrin - fibrin deposition; PMN - polymorphonuclear cell infiltrate

The results indicate the synergistic effects of using a complement inhibiting drug in addition to an immunosuppressant. In untreated mice the grafts were rejected in about 8 davs. Use of the immunosuppressant cyclosporin A alone on a daily, chronic basis resulted

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in an increase in graft survival until approximately 15 days post-transplant. The use of the anti-C5 antibody BB5.1 to inhibit formation of terminal complement had no effect on its own, graft rejection occurring at 8 days post-transplant as in the control group (Group 1'). The combination of BB5.1 through day 28 post-transplant plus cyclosporin A showed a synergistic effect with graft survival being extended until approximately day 80. A more surprising result is that of Group 5' in which BB5.1 and cyclosporin A were each administered chronically post-transplant. In this case the graft survival was for more than 100 days (as much data as presently available). Additionally, the histological results shown in Table 4 indicate that the administration of both BB5.1 and cyclosporin A protected the graft from changes much better than either BB5.1 or cyclosporin A alone, and that the chronic administration of BB5.1 and cyclosporin A protected the graft to such an extent that even at 100 days post-transplant there were no histological changes seen in the engrafted hearts. A survival time of 100 days in these models is considered to be the gold standard. A survival of 100 days in the model is believed to indicate that there will be an indefinite survival of the allograft. When BB5.1 administration was stopped after 28 days, the grafts were protected but they did begin to show some minimal to mild histological changes by about day 80 which was the time at which graft failure occurred.

The Group 4' mice were treated differently in that they were administered BB5.1 on a daily basis by an intravenous administration. These animals became ill, showing weight loss and urine retention and were sacrificed at a time at which the grafted hearts were still beating although their function had declined. This was the first group of mice studied and it is unknown why these ill effects were seen. These ill effects were not seen when the BB5.1 was administered intraperitoneally with a schedule of three times per week. As seen below in Example 4, daily administration of BB5.1 via an intraperitoneal route did not cause ill effects. Also, intravenous administration was not necessarily the cause of the illness in these animals. Intravenous administration of eculizumab (a human equivalent antibody to BB5.1 in that it binds to human C5) has been successfully administered intravenously without ill effects to humans in a study of PNH (Hillmen et al., 2004). Complement inhibitors may be administered by other routes in addition to intravenous and intraperitoneal, with all such routes being well known by those skilled in the art.

Example 4

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Accelerated Rejection in a Presensitized Heart Transplantation Model

A second set of experiments similar to those of Example 3 was performed but the recipient mouse was presensitized to the donor organ. In these experiments, the presensitization was brought about by prior transplantation of a skin graft. In general, presensitization can occur not only as a result of having received an earlier allograft, but can also be caused by having received multiple blood transfusions or in women who have been pregnant. Besides such presensitization methods, allografts with an ABO mismatch will be rapidly attacked and rejected because of preformed antibodies to the ABO antigens unless steps are taken to prevent such an attack.

Some mice in these studies were administered cyclophosphamide in addition to BB5.1 and/or cyclosporin A. For these experiments BALB/c recipient mice were presensitized with C3H skin grafts one week prior to heart transplantation from the same donor (using the method of Pruitt and Bollinger, 1991). This model is designed to mimic presensitized transplantation in humans, especially in relation to accelerated humoral rejection. Recipient mice were split into eight groups of six to eight mice each. The treatments were as follow.

Group 1" (control) - mice (male 8-12 week old mice weighing 25 – 30 g) were administered 0.75 mL saline intraperitoneally on a daily basis beginning at day -1 and continuing until the endpoint (graft rejection).

Group 2" (cyclosporin A alone) - mice were administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight beginning on day 0 (day of transplant) until the endpoint.

Group 3" (BB5.1 alone) - mice were administered the anti-mouse complement monoclonal antibody BB5.1 at a dose of 40 mg/kg body weight delivered intraperitoneally on a daily basis beginning at day -1 and continuing until the endpoint.

Group 4" (cyclophosphamide alone) - mice were administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 5" (BB5.1 plus cyclosporin A) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until the endpoint. These mice were additionally administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until the endpoint.

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Group 6" (BB5.1 plus cyclophosphamide) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until the endpoint. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 7" (cyclosporin A plus cyclophosphamide) - mice were administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until the endpoint. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 8" (BB5.1 plus cyclosporin A plus cyclophosphamide) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until 100 days. These mice were also administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until 100 days. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1. Two mice in this group were sacrificed at day 60 for histological studies (no rejection had yet occurred) and the four remaining mice still had not rejected their grafts by day 100.

Additionally a control group of mice which was not presensitized and received only the saline treatment as for Group 1" was tested.

The results of these experiments are shown in Tables 5 and 6. Table 5 lists survival times for the grafts and Table 6 summarizes the histological results.

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Table 5

Allograft Survival

Groups (Treatment)	Individual survival (days)	Mean survival time (days)
No presensitization	8, 8, 8, 8, 8, 9	8.3 ± 0.5*
1". One skin presensitization	3, 3, 3, 3, 3, 3, 3, 4	3.1 ± 0.4
2". Cyclosporin A	3, 3, 3, 3	3.0 ± 0.0
3". BB5.1	3, 3, 4, 4	3.5 ± 0.6
4". Cyclophosphamide	3, 3, 3, 4	3.3 ± 0.5
5". BB5.1 + Cyclosporin A	10, 10, 11, 11, 12, 12, 14, 15	11.9 ± 1.8**
6". BB5.1 + Cyclophosphamide	3, 3, 3, 3, 3, 4	3.2 ± 0.4
7". Cyclosporin A + Cyclophosphamide	3, 3, 3, 4, 4, 4	3.5 ± 0.6
8". BB5.1 + Cyclosporin A + Cyclophosphamide	> 100 days (4 mice)	> 100***

*P < 0.01 group 1" vs. no presensitization

**P < 0.01 group 5" vs. groups 1"-4" and 6"-7".

***P<0.01 group 8" vs. groups 1"-7".

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Table 6

Median Scores of Histological Changes of Heart Allografts at Necropsy

Groups	Vasc*	Infar	Lymph	Throm	Hemo	Fibrin	PMN
No presensitization (endpoint)	3.0	3.0	1.0	4.0	3.0	3.0	3.0
1". One skin presensitization (endpoint)	0.0	4.0	1.0	4.0	3.0	0.0	0.0
2". Cyclosporin A (endpoint)	0.0	4.0	0.0	3.0	3.0	N/A	N/A
3". BB5.1 (endpoint)	2.0	2.0	2.0	2.0	3.0	N/A	N/A
4". Cyclophosphamide (endpoint)	2.0	4.0	0.0	3.0	2.0	N/A	N/A
5". BB5.1 + Cyclosporin A (post-operative day 3)	0.0	1.0	1.0	0.0	0.0	0.0	0.0
5". BB5.1 + Cyclosporin A (endpoint)	2.0	2.0	2.0	2.0	2.0	0.0	0.0
6". BB5.1 + Cyclophosphamide (endpoint)	1.0	3.0	0.0	3.0	2.0	N/A	N/A
7". Cyclosporin A + Cyclophosphamide (endpoint)	0.0	1.0	1.0	2.0	3.0	N/A	N/A
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 3)	0.0	0.0	1.0	0.0	0.0	N/A	N/A
8. BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 12)	0.0	0.0	1.0	0.0	0.0	N/A	N/A
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-	0.0	0.0	1.0	0.0	0.0	N/A	N/A

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operative day 60)							
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 100)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Median scores: 0 - normal; 1- minimum change; 2 - mild change; 3 - moderate change; 4 - marked change. N/A - not available.

*Vasc - vasculitis; Infar - infarction; Lymph - lymphocyte infiltration; Throm - thrombosis; Hemo - hemorrhage; Fibrin - fibrin deposition; PMN - polymorphonuclear cell infiltrate

The results shown in Table 5 indicate a difference between the presensitized mouse model and the nonpresensitized mouse model as used in Example 3. The results indicate that in the absence of presensitization, grafts are rejected in approximately 8 days in the absence of treatment with any drugs. Presensitizing the animals causes a more rapid rejection, the rejection of the graft in the presensitized animals being in approximately 3 days in the absence of any drug treatment. Treatment with either BB5.1, cyclosporin A or cyclophosphamide had no effect upon graft survival, with the grafts being rejected in approximately 3-4 days in each of these groups of animals. The combination of BB5.1 and cyclosporin A showed some effect with rejection occurring about day 12. The combination of BB5.1 and cyclophosphamide had no protective effect with rejection occurring about day 3. Similarly the combination of cyclosporin A and cyclophosphamide had essentially no protective effect with rejection occurring at 3-4 days. Very surprisingly, the combination of all three drugs (chronic administration of BB5.1 and cyclosporin plus administration of cyclophosphamide at the time of transplant) showed a highly synergistic effect with all of the mice surviving for more than 100 days. Again, a survival of 100 days in this model is considered to be the gold standard and assumes an indefinite survival.

These results as well as the histological results as shown in Table 6 indicate that the combination of chronic treatment with a complement inhibitor and an immunosuppressant such as cyclosporin A in treating a presensitized mouse results in some attenuation of accelerated rejection. Treatment of these animals additionally with cyclophosphamide at the time of transplant and on the first day after transplant results in a much greater time of survival, no rejection having been seen by at least day 100.

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It will be appreciated that the methods and compositions of the instant disclosure can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the disclosure. Thus, the described embodiments are illustrative and should not be construed as restrictive.

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LIST OF REFERENCES

The publications and other materials used herein to illuminate the background of the disclosure, and in particular, cases to provide additional details respecting the practice, are incorporated herein by reference in their entirety, and for convenience, are referenced by author and date in the text and respectively grouped in the following List of References.

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The contents of all references cited herein are incorporated by reference in their entirety.

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CLAIMS

1. A method to prolong survival of an MHC mismatched allograft in a recipient mammal, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

2. The method of claim 1 wherein said mammal is a human.

3. The method of claim 2 wherein said MHC mismatched allograft is an HLA mismatched allograft.

4. The method of claim 1 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

5. The method of claim 4 wherein said drug which inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

6. The method of claim 5 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

7. The method of claim 5 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

8. The method of claim 5 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

9. The method of claim 5 wherein said antibody fragment is pexelizumab.

10. The method of claim 5 wherein said whole antibody is eculizumab.

11. The method claim 10 wherein said eculizumab is administered once every 2 weeks.

12. The method of claim 1 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

13. The method of claim 1 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

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14. The method of claim 1 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

15. The method of claim 1 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

16. The method of claim 1 wherein more than one immunosuppressive drug is administered.

17. The method of claim 1 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

18. The method of claim 17 wherein said drug which inhibits complement activity is an antibody which inhibits cleavage of complement C5.

19. The method of claim 1 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

20. The method of claim 1 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

21. The method of claim 1 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

22. The method of claim 1 wherein said allograft survives for the remaining life-time of said mammal.

23. The method of claim 2 wherein said allograft survives for at least six months.

24. The method of claim 2 wherein said allograft survives for at least one year.

25. The method of claim 2 wherein said allograft survives for at least five years.

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26. The method of claim 2 wherein said allograft survives for the remaining life-time of said human.

27. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

28. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

29. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

30. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

31. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

32. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

33. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

34. The method of claim 1 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

35. The method of claim 34 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

36. A method to prolong survival of an allograft in a recipient mammal presensitized to said allograft, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

37. The method of claim 36 wherein said mammal is a human.

38. The method of claim 36 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

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39. The method of claim 38 wherein said drug that inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

40. The method of claim 39 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

41. The method of claim 39 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

42. The method of claim 39 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

43. The method of claim 39 wherein said antibody fragment is pexelizumab.

44. The method of claim 39 wherein said whole antibody is eculizumab.

45. The method claim 44 wherein said eculizumab is administered once every 2 weeks.

46. The method of claim 36 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

47. The method of claim 36 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

48. The method of claim 36 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

49. The method of claim 36 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

50. The method of claim 36 wherein more than one immunosuppressive drug is administered.

51. The method of claim 36 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

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52. The method of claim 51 wherein said method comprises administering i) a drug which inhibits complement activity, ii) cyclosporin A, and iii) cyclophosphamide.

53. The method of claim 51 wherein said drug which inhibits complement activity is an antibody which inhibits cleavage of complement C5.

54. The method of claim 36 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

55. The method of claim 36 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

56. The method of claim 36 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

57. The method of claim 36 wherein said allograft survives for the remaining life-time of said mammal.

58. The method of claim 37 wherein said allograft survives for at least six months.

59. The method of claim 37 wherein said allograft survives for at least one year.

60. The method of claim 37 wherein said allograft survives for at least five years.

61. The method of claim 37 wherein said allograft survives for the remaining life-time of said human.

62. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

63. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

64. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

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65. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

66. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

67. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

68. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

69. The method of claim 36 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

70. The method of claim 69 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

71. A method to prolong survival of an allograft in a recipient mammal which is an ABO mismatch to said allograft, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

72. The method of claim 71 wherein said mammal is a human.

73. The method of claim 71 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

74. The method of claim 73 wherein said drug which inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

75. The method of claim 74 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

76. The method of claim 74 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

77. The method of claim 74 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

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78. The method of claim 74 wherein said antibody fragment is pexelizumab.

79. The method of claim 74 wherein said whole antibody is eculizumab.

80. The method claim 79 wherein said eculizumab is administered once every 2 weeks.

81. The method of claim 71 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

82. The method of claim 71 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

83. The method of claim 71 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

84. The method of claim 71 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

85. The method of claim 71 wherein more than one immunosuppressive drug is administered.

86. The method of claim 71 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

87. The method of claim 86 wherein said method comprises administering i) a drug which inhibits complement activity, ii) cyclosporin A, and iii) cyclophosphamide.

88. The method of claim 86 wherein said drug that inhibits complement activity is an antibody that inhibits cleavage of complement C5.

89. The method of claim 71 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

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90. The method of claim 71 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug that inhibits complement activity.

91. The method of claim 71 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug that inhibits complement activity.

92. The method of claim 71 wherein said allograft survives for the remaining life-time of said mammal.

93. The method of claim 72 wherein said allograft survives for at least six months.

94. The method of claim 72 wherein said allograft survives for at least one year.

95. The method of claim 72 wherein said allograft survives for at least five years.

96. The method of claim 72 wherein said allograft survives for the remaining life-time of said human.

97. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

98. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

99. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

100. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

101. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

102. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

103. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

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104. The method of claim 71 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

105. The method of claim 104 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

106. Use of a drug that inhibits complement activity and an immunosuppressive drug in the manufacture of a medicament or medicament package for prolong survival of an allograft in a mammal.

107. The use of claim 106, wherein more than one immunosuppressive drug is included in said medicament or medicament package.

108. The use of claim 106, wherein said drug that inhibits complement activity and said immunosuppressive drug are in a formulation suitable for concurrent administration to said mammal.

109. The use of claim 106, wherein said drug that inhibits complement activity and said immunosuppressive drug are in a formulation or formulations suitable for sequential administration to said mammal.

110. The use of claim 106, wherein said drug that inhibits complement activity is in a formulation suitable for chronic administration to said mammal.

111. The use of claim 106, wherein said immunosuppressive drug is in a formulation suitable for chronic administration to said mammal.

112. A method to prolong survival of allograft in a recipient mammal, comprising preparing an allograft from a first allograft accommodated by a first recipient mammal, wherein said first recipient mammal, after receiving said first allograft, has been treated with a drug that inhibits complement activity and at least one immunosuppressive agent.

113. An allograft that has prolonged survival in a recipient mammal, wherein said allograft is prepared from a first recipient mammal and was transplanted to the first recipient mammal that has been treated with a drug that inhibits complement activity and at least one immunosuppressive agent.

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114. A pharmaceutical package comprising a drug that inhibits complement activity and at least one immunosuppressive agent, wherein said drug and said agent are formulated for chronic administration.

115. The pharmaceutical package of claim 114, wherein said drug is an antibody in a lyophilized formulation comprising the antibody and a lyoprotectant.

116. The pharmaceutical package of claim 114, wherein said agent is in a lyophilized formulation comprising the agent and a lyoprotectant.

117. The pharmaceutical package of claim 114, wherein said drug and said agent are in the same lyophilized formulation comprising said drug, said agent, and a lyoprotectant.

118. The pharmaceutical package of claim 114, wherein said drug is in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said drug in a formulation suitable for injection.

119. The pharmaceutical package of claim 114, wherein said agent is in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said agent in a formulation suitable for injection.

120. The pharmaceutical package of claim 114, wherein said drug and said agent are in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said drug and said agent in a formulation suitable for injection.

121. The pharmaceutical package of claim 114, wherein said drug is present in unit dosage form.

122. The pharmaceutical package of claim 114, wherein said agent is present in unit dosage form.

123. A lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant, wherein said antibody formulation is suitable for chronic administration.

124. The lyophilized antibody formulation of claim 123, wherein the antibody inhibits the formation of terminal complement or C5a.

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125. The lyophilized antibody formulation of claim 123, wherein the antibody is a whole antibody or an antibody fragment.

126. The lyophilized antibody formulation of claim 125, wherein the antibody wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

127. The lyophilized antibody formulation of claim 125, wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

128. The lyophilized antibody formulation of claim 125, wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

129. The lyophilized antibody formulation of claim 125, wherein said antibody fragment is pexelizumab.

130. The lyophilized antibody formulation of claim 125, wherein said whole antibody is eculizumab.

131. An injection system comprising a syringe, wherein said syringe comprises a cartridge containing an antibody that inhibits complement activity and is formulated suitable for injection.

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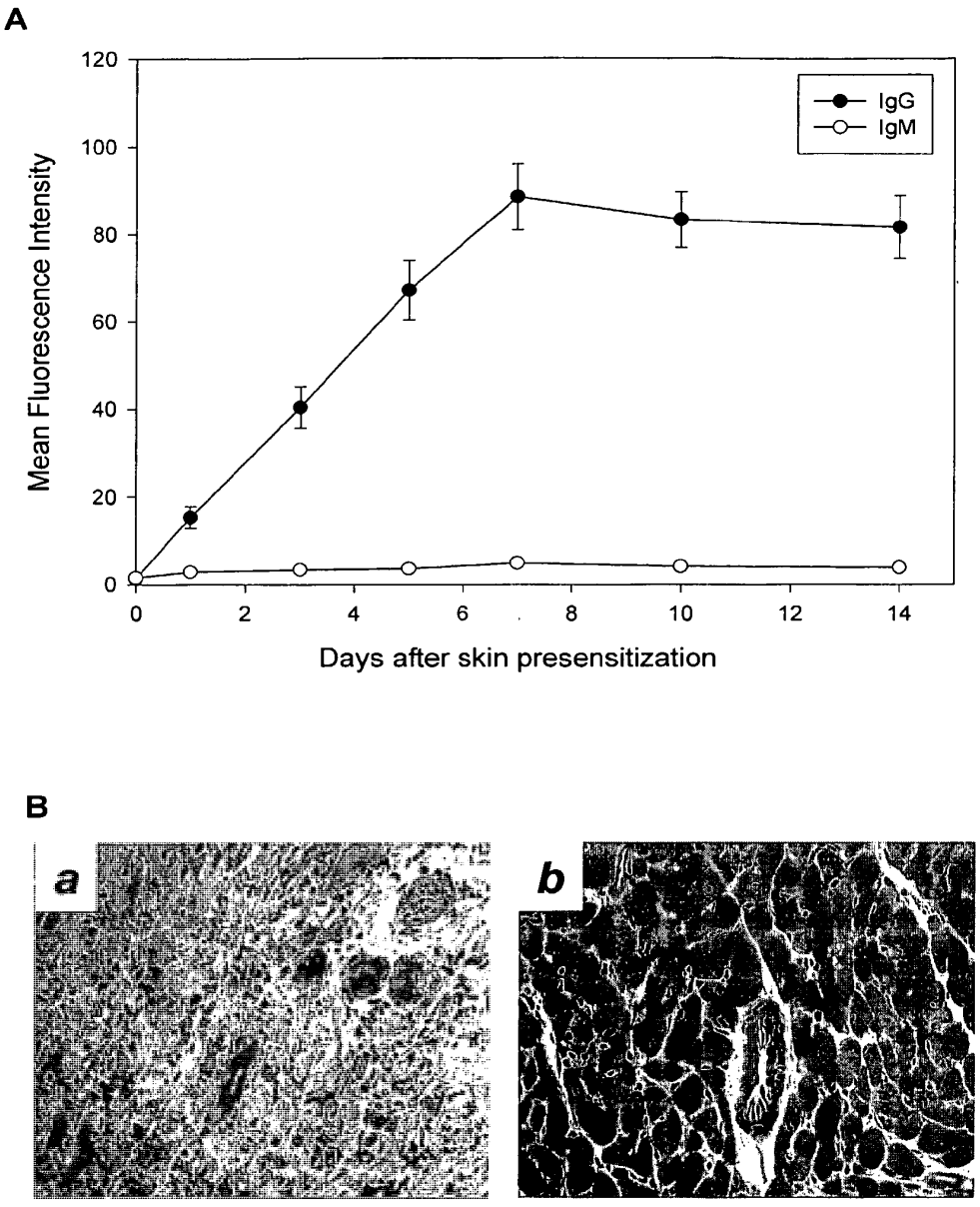
A
120
100
80
60
40
20
0
Mean Fluorescence Intensity
IgG
IgM
0
2
4
6
8
10
12
14
Days after skin presensitization
B
a
b

Figure 1A, B

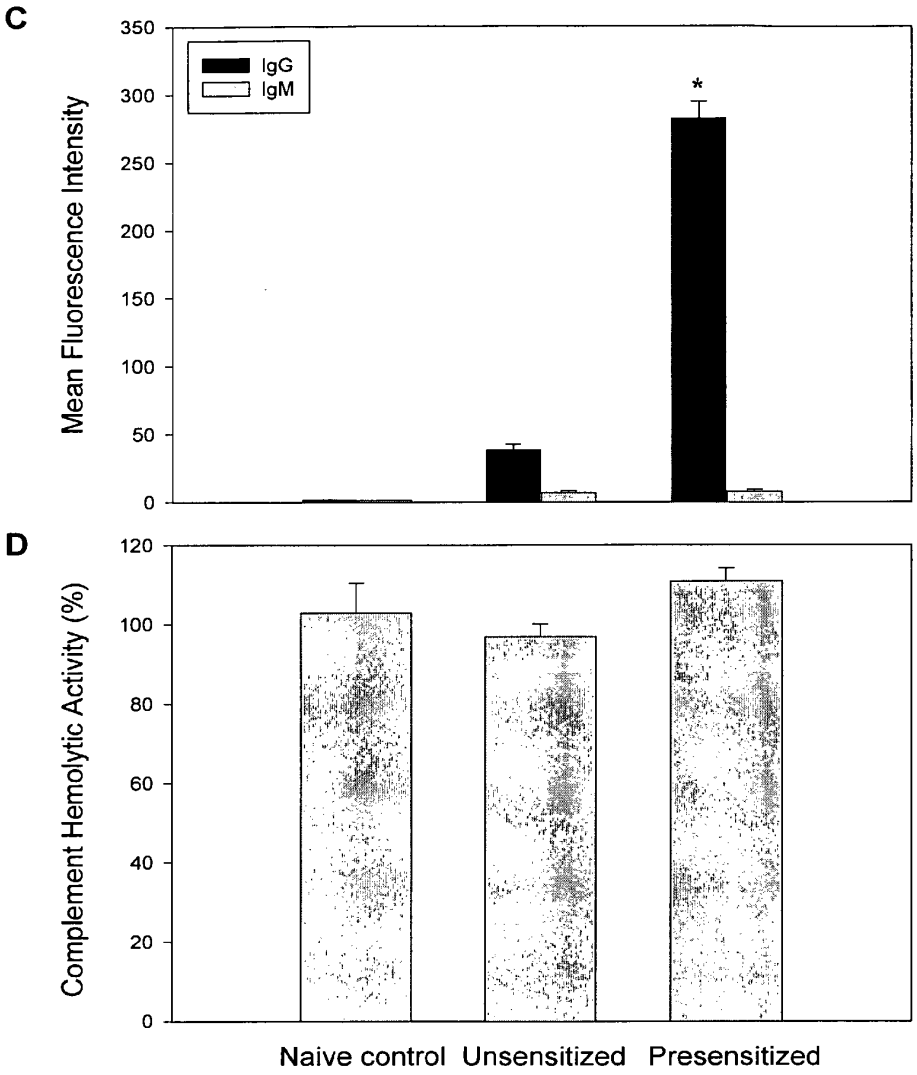

Figure 1C, D

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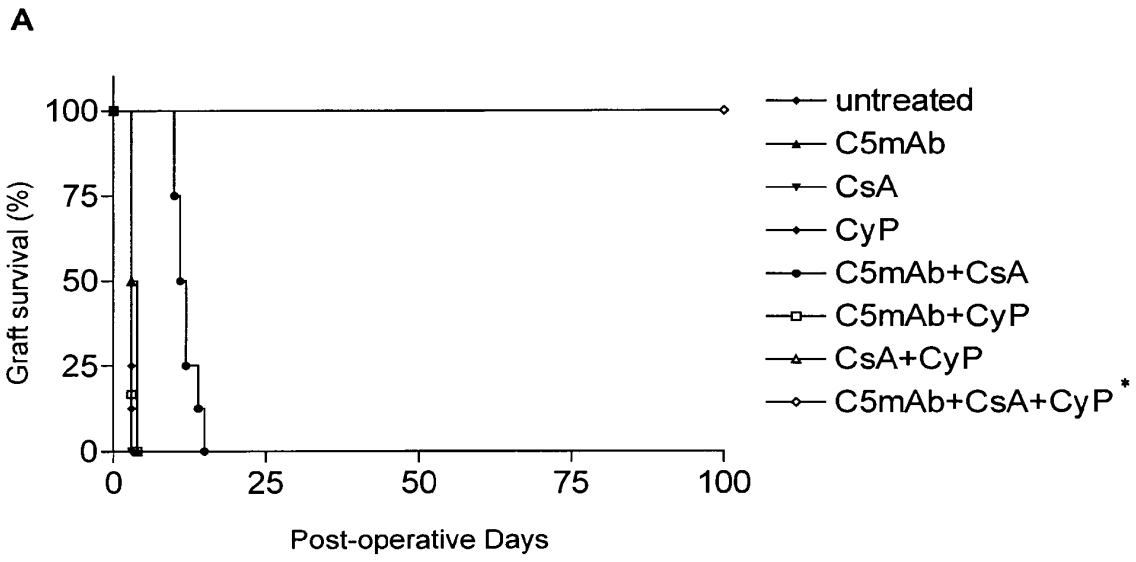
A
Graft survival (%)
100
75
50
25
0
0
25
50
75
100
Post-operative Days
untreated
C5mAb
CsA
CyP
C5mAb+CsA
C5mAb+CyP
CsA+CyP
C5mAb+CsA+CyP *

Figure 2A

B

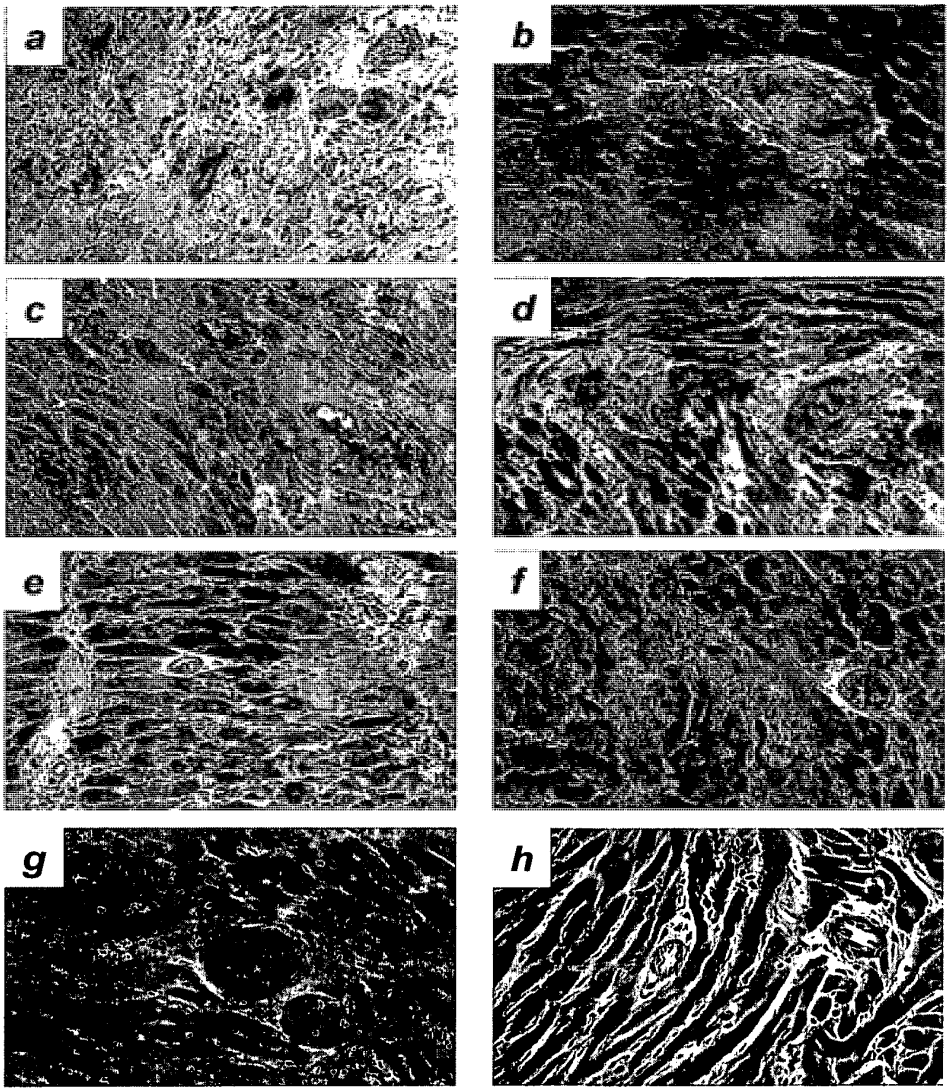

Figure 2B

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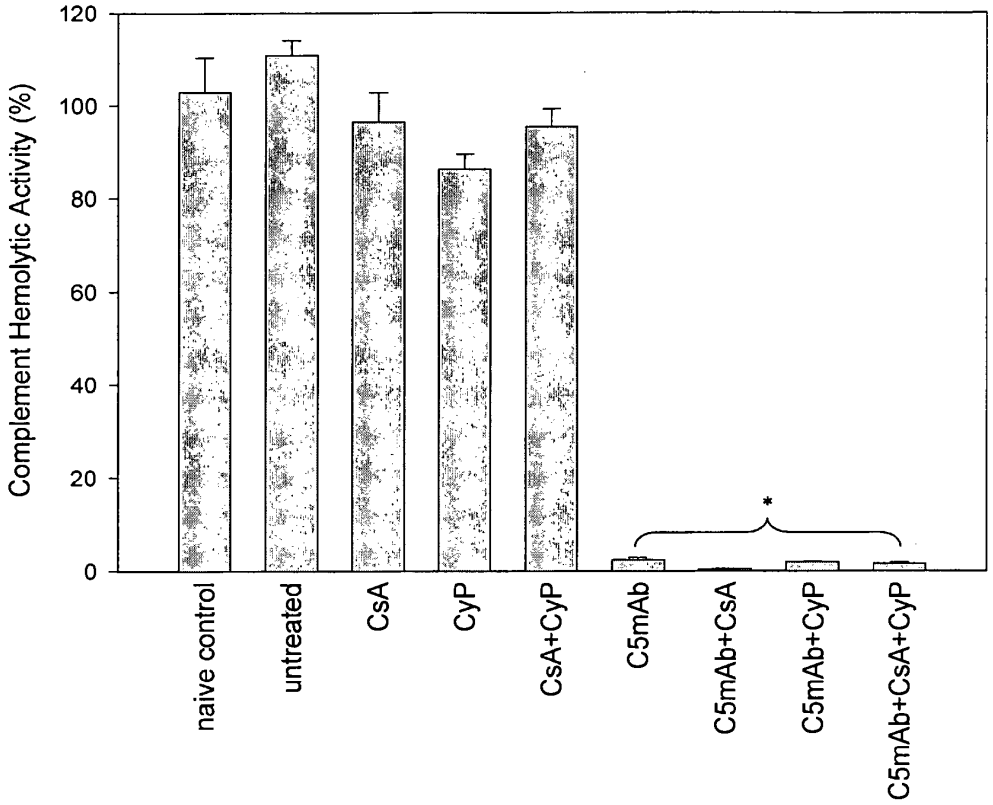
Complement Hemolytic Activity (%)
120
100
80
60
40
20
0
*
naive control
untreated
CsA
CyP
CsA+CyP
C5mAb
C5mAb+CsA
C5mAb+CyP
C5mAb+CsA+CyP

Figure 3

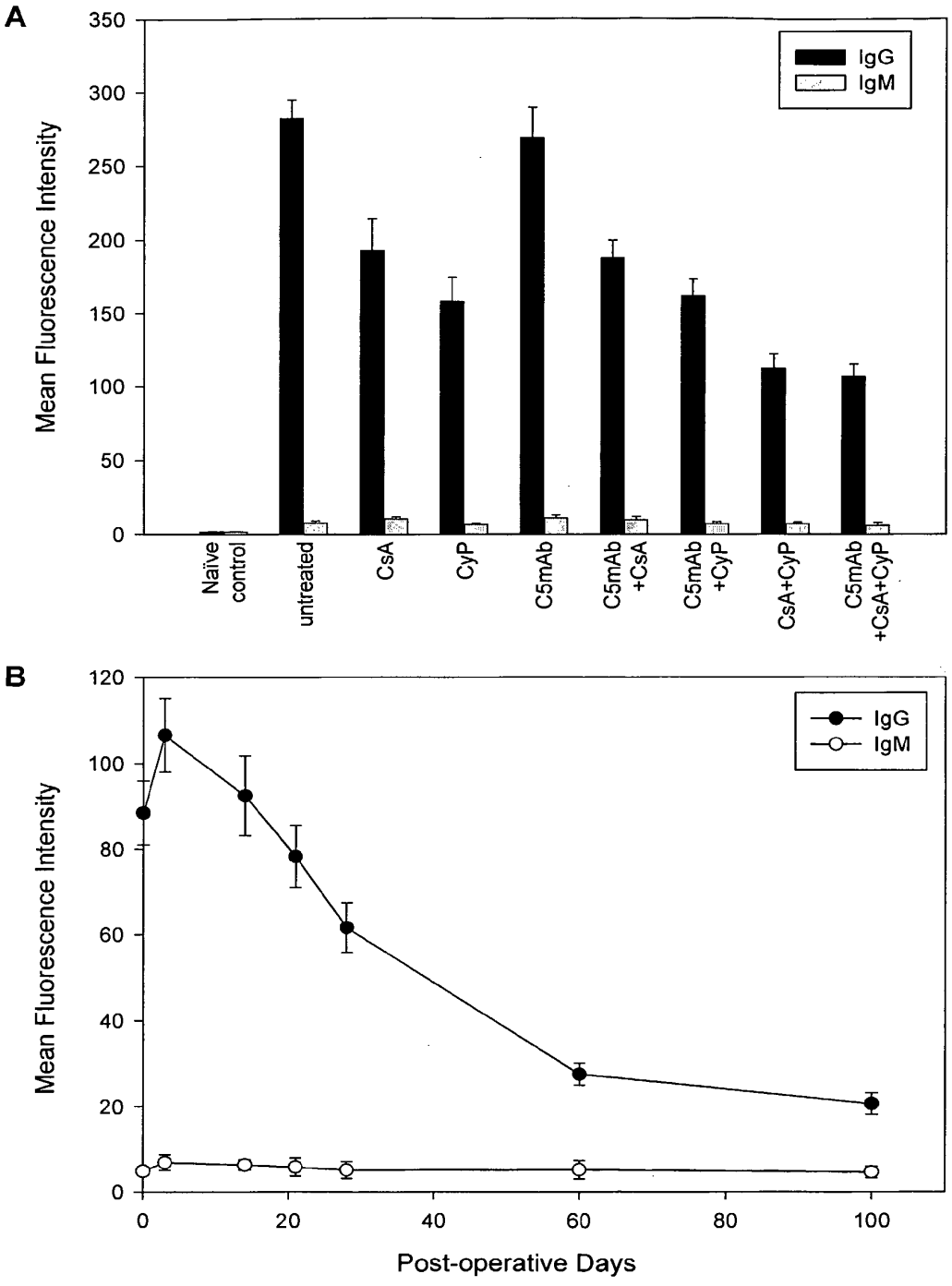

Figure 4A, B

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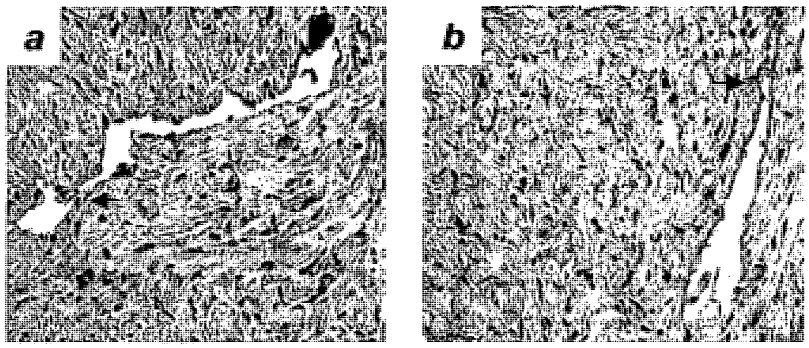
C



D

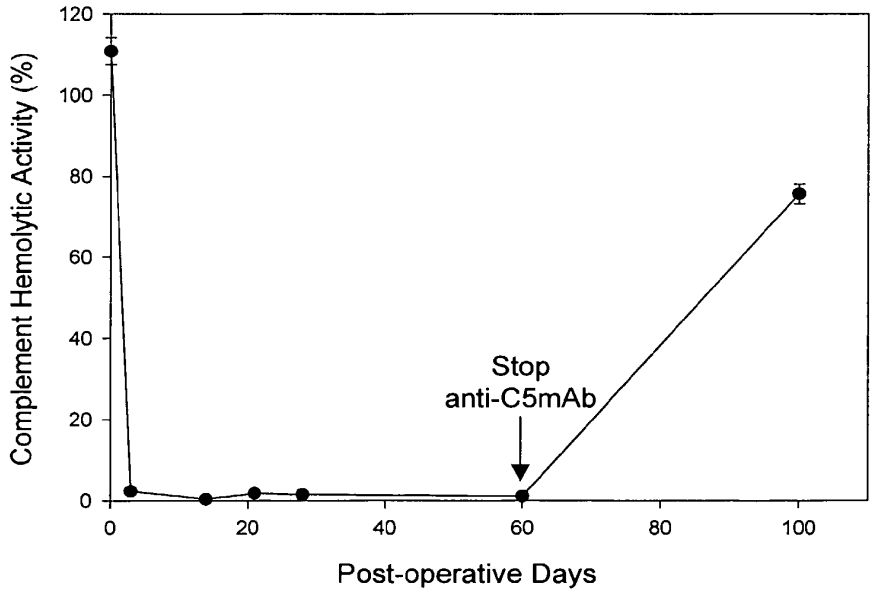


Figure 4C, D

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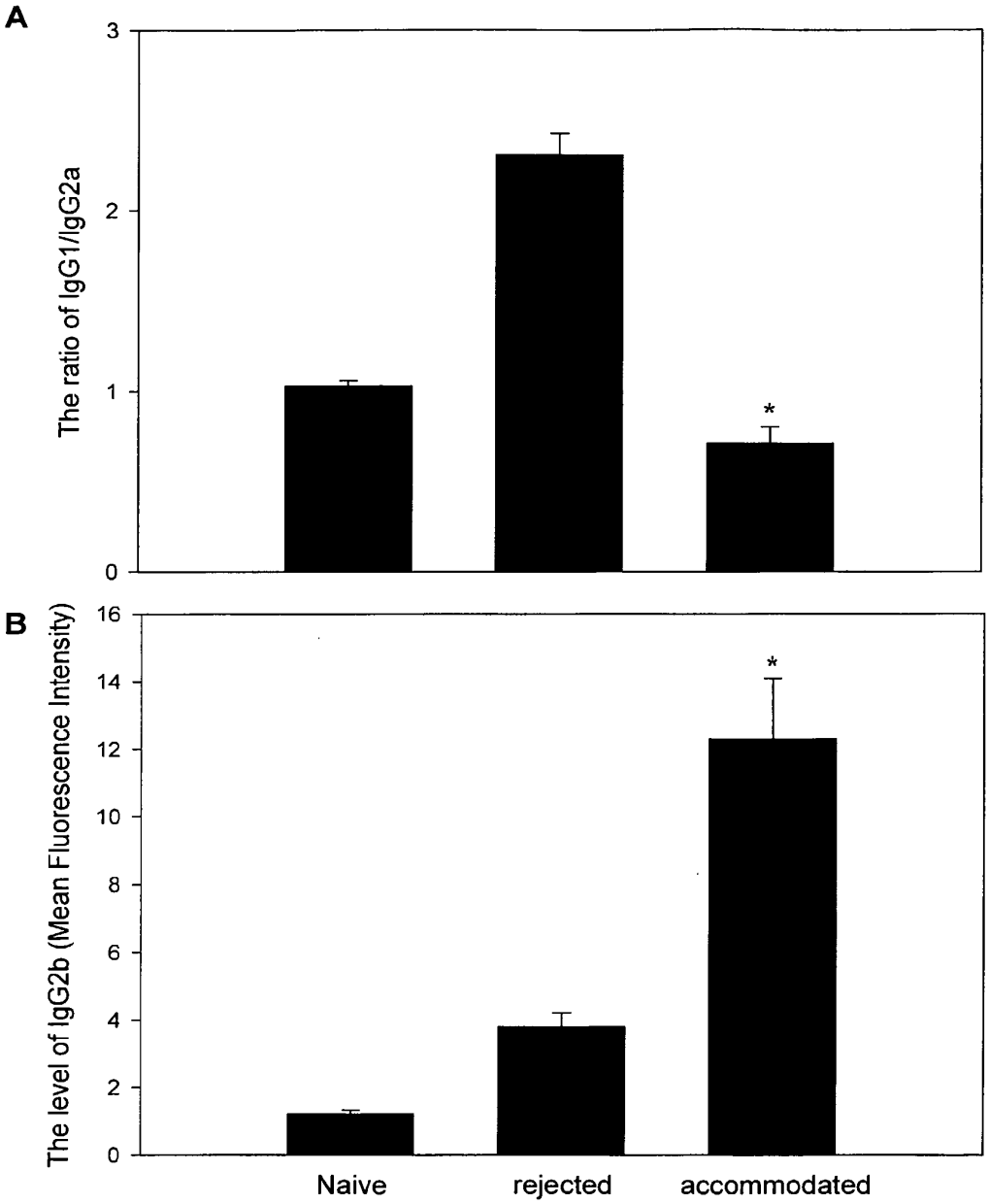

Figure 5A, B

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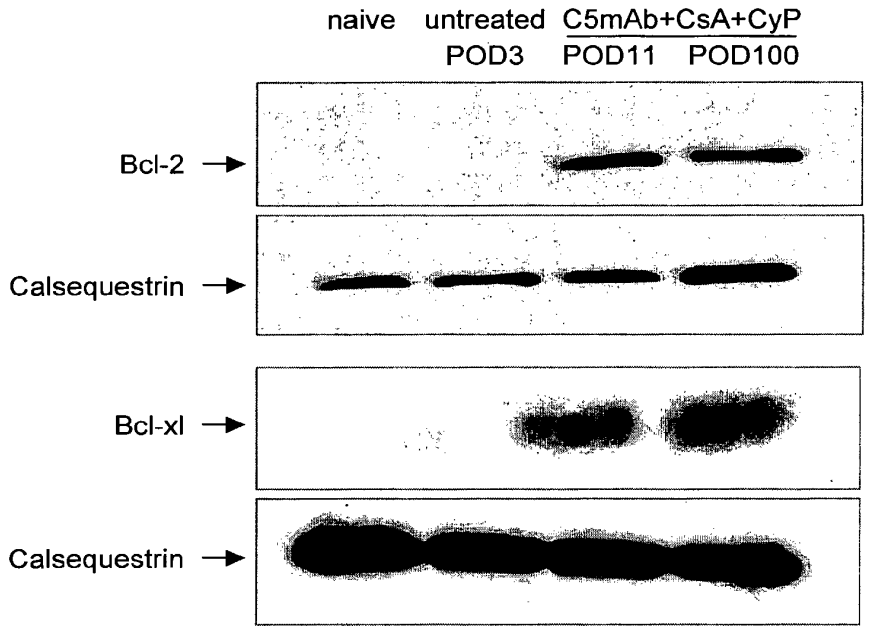

Figure 6

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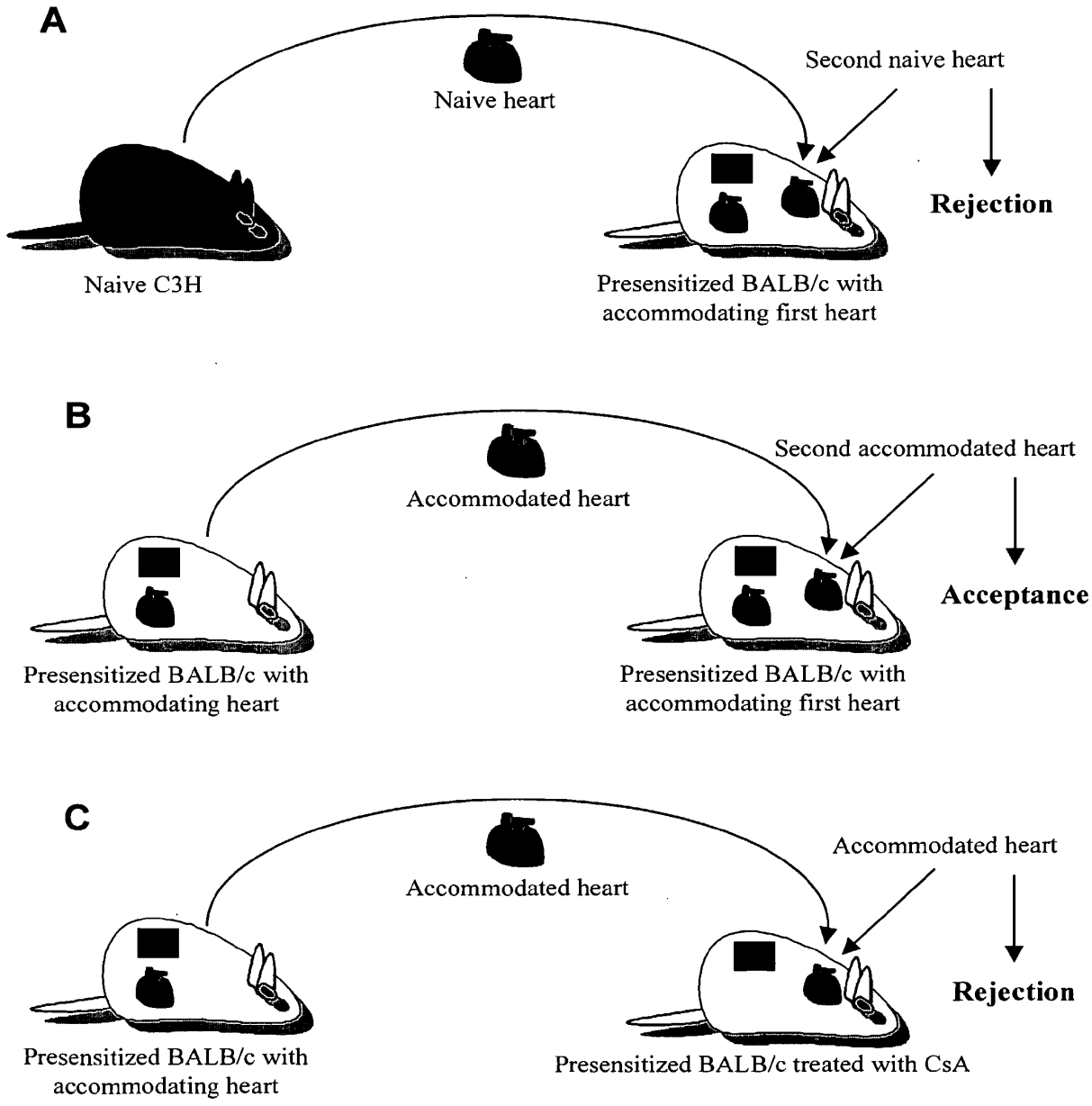

Figure 7

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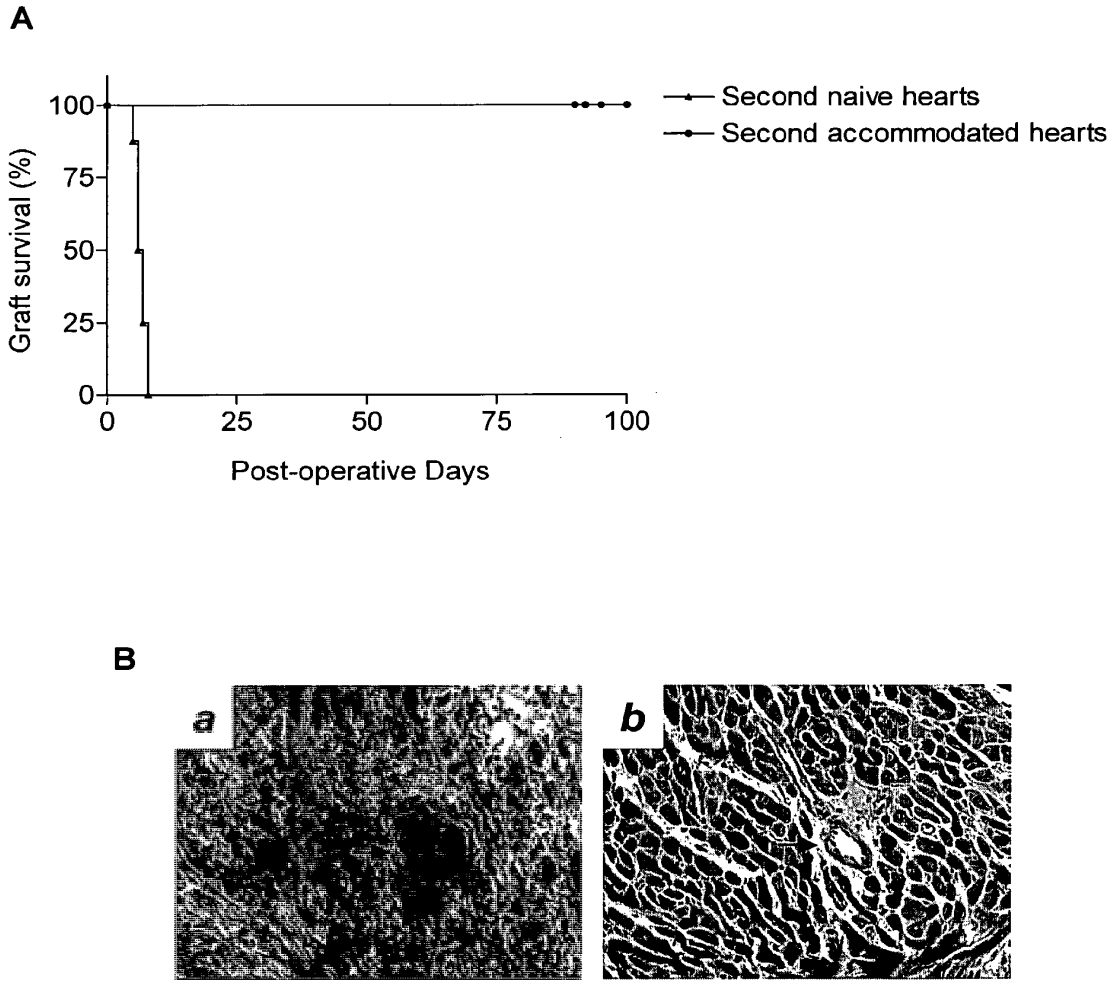
A
100
75
50
25
0
Graft survival (%)
0
25
50
75
100
Post-operative Days
Second naive hearts
Second accommodated hearts
B
a
b

Figure 8

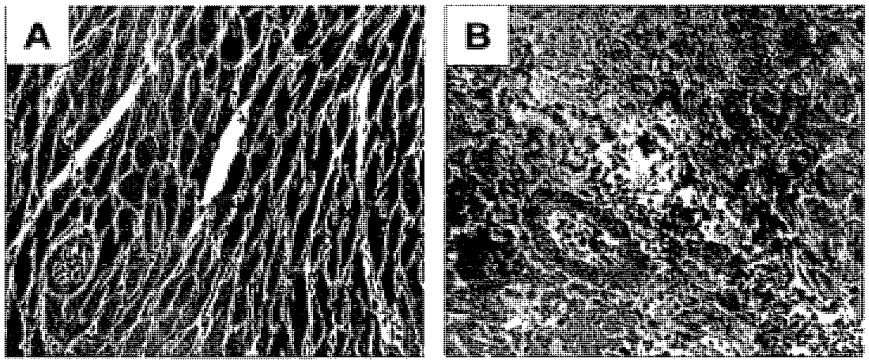

Figure 9

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(71) Applicant *(for all designated States except US)*: **XENCOR** [US/US]; 111 West Lemon Avenue, Monrovia, CA 91016 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LAZAR, Gregory, Alan** [US/US]; 268 W. Dryden Street, #114, Glendale, CA 91202 (US). **CHIRINO, Arthur, J.** [US/US]; 6816 Pueblo Vista, Camarillo, CA 91106 (US). **DANG, Wei** [CN/US]; 1111 Blanche Street, Apt. 312, Pasadena, CA 91106 (US). **DESJARLAIS, John, Rudolph** [US/US]; 2096 E. Crary Street, Pasadena, CA 91104 (US). **DOBERSTEIN, Stephen, Kohn** [US/US]; 700 S. Lake Avenue, #202, Pasadena, CA 91106 (US). **HAYES, Robert, J.** [GB/US]; 190 Arroyo Terrace, Pasadena, CA 91103 (US). **KARKI, Sher, Basudur** [NP/US]; 300 North El Molino Avenue, Pasadena, CA 91101 (US). **VAFA, Omid** [IR/US]; 724 Ocean View Avenue, Monrovia, CA 91016 (US).

(74) Agents: **SILVA, Robin, M.** et al.; DORSEY & WHITNEY LLP, 4 Embarcadero Center, Suite 3400, San Francisco, CA 94111 (US).

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(54) Title: OPTIMIZED FC VARIANTS AND METHODS FOR THEIR GENERATION

(57) Abstract: The present invention relates to optimized Fc variants, methods for their generation, and antibodies and Fc fusions comprising optimized Fc variants.

OPTIMIZED Fc VARIANTS AND METHODS FOR THEIR GENERATION

[001] This application claims benefit under 35 U.S.C. §199(e) to USSNs 60/477,839 filed June 12, 2003; 60/467,606, filed May 2, 2003; 60/414,433 filed September 27, 2002; and 60/442,301 filed January 23, 2003, all of which are expressly incorporated by reference in their entirety.

FIELD OF THE INVENTION

[002] The present invention relates to novel optimized Fc variants, engineering methods for their generation, and their application, particularly for therapeutic purposes.

BACKGROUND OF THE INVENTION

[003] Antibodies are immunological proteins that bind a specific antigen. In most mammals, including humans and mice, antibodies are constructed from paired heavy and light polypeptide chains. Each chain is made up of individual immunoglobulin (Ig) domains, and thus the generic term immunoglobulin is used for such proteins. Each chain is made up of two distinct regions, referred to as the variable and constant regions. The light and heavy chain variable regions show significant sequence diversity between antibodies, and are responsible for binding the target antigen. The constant regions show less sequence diversity, and are responsible for binding a number of natural proteins to elicit important biochemical events. In humans there are five different classes of antibodies including IgA (which includes subclasses IgA1 and IgA2), IgD, IgE, IgG (which includes subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. The distinguishing features between these antibody classes are their constant regions, although subtler differences may exist in the V region. Figure 1 shows an IgG1 antibody, used here as an example to describe the general structural features of immunoglobulins. IgG antibodies are tetrameric proteins composed of two heavy chains and two light chains. The IgG heavy chain is composed of four immunoglobulin domains linked from N- to C-terminus in the order V_H-Cγ1-Cγ2-Cγ3, referring to the heavy chain variable domain, constant gamma 1 domain, constant gamma 2 domain, and constant gamma 3 domain respectively. The IgG light chain is composed of two immunoglobulin domains linked from N- to C-terminus in the order V_L-C_L, referring to the light chain variable domain and the light chain constant domain respectively.

[004] The variable region of an antibody contains the antigen binding determinants of the molecule, and thus determines the specificity of an antibody for its target antigen. The variable region is so named because it is the most distinct in sequence from other antibodies within the same class. The majority of sequence variability occurs in the complementarity determining regions (CDRs). There are 6 CDRs total, three each per heavy and light chain, designated V_H CDR1, V_H CDR2, V_H CDR3, V_L

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CDR1, V_L CDR2, and V_L CDR3. The variable region outside of the CDRs is referred to as the framework (FR) region. Although not as diverse as the CDRs, sequence variability does occur in the FR region between different antibodies. Overall, this characteristic architecture of antibodies provides a stable scaffold (the FR region) upon which substantial antigen binding diversity (the CDRs) can be explored by the immune system to obtain specificity for a broad array of antigens. A number of high-resolution structures are available for a variety of variable region fragments from different organisms, some unbound and some in complex with antigen. The sequence and structural features of antibody variable regions are well characterized (Morea *et al.*, 1997, *Biophys Chem* 68:9-16; Morea *et al.*, 2000, *Methods* 20:267-279), and the conserved features of antibodies have enabled the development of a wealth of antibody engineering techniques (Maynard *et al.*, 2000, *Annu Rev Biomed Eng* 2:339-376). For example, it is possible to graft the CDRs from one antibody, for example a murine antibody, onto the framework region of another antibody, for example a human antibody. This process, referred to in the art as "humanization", enables generation of less immunogenic antibody therapeutics from nonhuman antibodies. Fragments comprising the variable region can exist in the absence of other regions of the antibody, including for example the antigen binding fragment (Fab) comprising V_H-Cγ1 and V_H-C_L, the variable fragment (Fv) comprising V_H and V_L, the single chain variable fragment (scFv) comprising V_H and V_L linked together in the same chain, as well as a variety of other variable region fragments (Little *et al.*, 2000, *Immunol Today* 21:364-370).

[005] The Fc region of an antibody interacts with a number of Fc receptors and ligands, imparting an array of important functional capabilities referred to as effector functions. For IgG the Fc region, as shown in Figure 1, comprises Ig domains Cγ2 and Cγ3 and the N-terminal hinge leading into Cγ2. An important family of Fc receptors for the IgG class are the Fc gamma receptors (FcγRs). These receptors mediate communication between antibodies and the cellular arm of the immune system (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ravetch *et al.*, 2001, *Annu Rev Immunol* 19:275-290). In humans this protein family includes FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2) (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65). These receptors typically have an extracellular domain that mediates binding to Fc, a membrane spanning region, and an intracellular domain that may mediate some signaling event within the cell. These receptors are expressed in a variety of immune cells including monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, and $\gamma\gamma$ T cells. Formation of the Fc/FcγR complex recruits these effector cells to sites of bound antigen, typically resulting in signaling events within the cells and important subsequent immune responses such as release of inflammation mediators, B cell activation, endocytosis, phagocytosis, and cytotoxic attack. The ability to mediate cytotoxic and phagocytic effector functions is a potential mechanism by which antibodies destroy targeted cells. The cell-mediated reaction wherein nonspecific cytotoxic

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cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell is referred to as antibody dependent cell-mediated cytotoxicity (ADCC) (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ghetie *et al.*, 2000, *Annu Rev Immunol* 18:739-766; Ravetch *et al.*, 2001, *Annu Rev Immunol* 19:275-290). The cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell is referred to as antibody dependent cell-mediated phagocytosis (ADCP). A number of structures have been solved of the extracellular domains of human FcγRs, including FcγRIIa (pdb accession code 1H9V)(Sondermann *et al.*, 2001, *J Mol Biol* 309:737-749) (pdb accession code 1FCG)(Maxwell *et al.*, 1999, *Nat Struct Biol* 6:437-442), FcγRIIb (pdb accession code 2FCB)(Sondermann *et al.*, 1999, *Embo J* 18:1095-1103); and FcγRIIIb (pdb accession code 1E4J)(Sondermann *et al.*, 2000, *Nature* 406:267-273.). All FcγRs bind the same region on Fc, at the N-terminal end of the Cγ2 domain and the preceding hinge, shown in Figure 2. This interaction is well characterized structurally (Sondermann *et al.*, 2001, *J Mol Biol* 309:737-749), and several structures of the human Fc bound to the extracellular domain of human FcγRIIIb have been solved (pdb accession code 1E4K)(Sondermann *et al.*, 2000, *Nature* 406:267-273.) (pdb accession codes 1IIS and 1IIX)(Radaev *et al.*, 2001, *J Biol Chem* 276:16469-16477), as well as has the structure of the human IgE Fc/Fc□RI□ complex (pdb accession code 1F6A)(Garman *et al.*, 2000, *Nature* 406:259-266).

[006] The different IgG subclasses have different affinities for the FcγRs, with IgG1 and IgG3 typically binding substantially better to the receptors than IgG2 and IgG4 (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65). All FcγRs bind the same region on IgG Fc, yet with different affinities: the high affinity binder FcγRI has a Kd for IgG1 of 10^{-8} M^{-1}, whereas the low affinity receptors FcγRII and FcγRIII generally bind at 10^{-6} and 10^{-5} respectively. The extracellular domains of FcγRIIIa and FcγRIIIb are 96% identical, however FcγRIIIb does not have a intracellular signaling domain. Furthermore, whereas FcγRI, FcγRIIa/c, and FcγRIIIa are positive regulators of immune complex-triggered activation, characterized by having an intracellular domain that has an immunoreceptor tyrosine-based activation motif (ITAM), FcγRIIb has an immunoreceptor tyrosine-based inhibition motif (ITIM) and is therefore inhibitory. Thus the former are referred to as activation receptors, and FcγRIIb is referred to as an inhibitory receptor. The receptors also differ in expression pattern and levels on different immune cells. Yet another level of complexity is the existence of a number of FcγR polymorphisms in the human proteome. A particularly relevant polymorphism with clinical significance is V158/F158 FcγRIIIa. Human IgG1 binds with greater affinity to the V158 allotype than to the F158 allotype. This difference in affinity, and presumably its effect on ADCC and/or ADCP, has been shown to be a significant determinant of the efficacy of the anti-CD20 antibody rituximab (Rituxan®, a registered trademark of IDEC Pharmaceuticals Corporation). Patients with the V158 allotype respond favorably to rituximab treatment; however, patients with the lower affinity F158 allotype respond poorly (Cartron *et al.*, 2002, *Blood* 99:754-758). Approximately 10-20% of humans are V158/V158

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homozygous, 45% are V158/F158 heterozygous, and 35-45% of humans are F158/F158 homozygous (Lehrnbecher *et al.*, 1999, *Blood* 94:4220-4232; Cartron *et al.*, 2002, *Blood* 99:754-758). Thus 80-90% of humans are poor responders, that is they have at least one allele of the F158 FcγRIIIa.

[007] An overlapping but separate site on Fc, shown in Figure 1, serves as the interface for the complement protein C1q. In the same way that Fc/FcγR binding mediates ADCC, Fc/C1q binding mediates complement dependent cytotoxicity (CDC). C1q forms a complex with the serine proteases C1r and C1s to form the C1 complex. C1q is capable of binding six antibodies, although binding to two IgGs is sufficient to activate the complement cascade. Similar to Fc interaction with FcγRs, different IgG subclasses have different affinity for C1q, with IgG1 and IgG3 typically binding substantially better to the FcγRs than IgG2 and IgG4 (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65). There is currently no structure available for the Fc/C1q complex; however, mutagenesis studies have mapped the binding site on human IgG for C1q to a region involving residues D270, K322, K326, P329, and P331, and E333 (Idusogie *et al.*, 2000, *J Immunol* 164:4178-4184; Idusogie *et al.*, 2001, *J Immunol* 166:2571-2575).

[008] A site on Fc between the Cγ2 and Cγ3 domains, shown in Figure 1, mediates interaction with the neonatal receptor FcRn, the binding of which recycles endocytosed antibody from the endosome back to the bloodstream (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ghetie *et al.*, 2000, *Annu Rev Immunol* 18:739-766). This process, coupled with preclusion of kidney filtration due to the large size of the full length molecule, results in favorable antibody serum half-lives ranging from one to three weeks. Binding of Fc to FcRn also plays a key role in antibody transport. The binding site for FcRn on Fc is also the site at which the bacterial proteins A and G bind. The tight binding by these proteins is typically exploited as a means to purify antibodies by employing protein A or protein G affinity chromatography during protein purification. Thus the fidelity of this region on Fc is important for both the clinical properties of antibodies and their purification. Available structures of the rat Fc/FcRn complex (Martin *et al.*, 2001, *Mol Cell* 7:867-877), and of the complexes of Fc with proteins A and G (Deisenhofer, 1981, *Biochemistry* 20:2361-2370; Sauer-Eriksson *et al.*, 1995, *Structure* 3:265-278; Tashiro *et al.*, 1995, *Curr Opin Struct Biol* 5:471-481) provide insight into the interaction of Fc with these proteins.

[009] A key feature of the Fc region is the conserved N-linked glycosylation that occurs at N297, shown in Figure 1. This carbohydrate, or oligosaccharide as it is sometimes referred, plays a critical structural and functional role for the antibody, and is one of the principle reasons that antibodies must be produced using mammalian expression systems. While not wanting to be limited to one theory, it is believed that the structural purpose of this carbohydrate may be to stabilize or solubilize Fc, determine a specific angle or level of flexibility between the Cγ3 and Cγ2 domains, keep the two Cγ2 domains from aggregating with one another across the central axis, or a combination of these.

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Efficient Fc binding to FcγR and C1q requires this modification, and alterations in the composition of the N297 carbohydrate or its elimination affect binding to these proteins (Umaña *et al.*, 1999, *Nat Biotechnol* 17:176-180; Davies *et al.*, 2001, *Biotechnol Bioeng* 74:288-294; Mimura *et al.*, 2001, *J Biol Chem* 276:45539-45547.; Radaev *et al.*, 2001, *J Biol Chem* 276:16478-16483; Shields *et al.*, 2001, *J Biol Chem* 276:6591-6604; Shields *et al.*, 2002, *J Biol Chem* 277:26733-26740; Simmons *et al.*, 2002, *J Immunol Methods* 263:133-147). Yet the carbohydrate makes little if any specific contact with FcγRs (Radaev *et al.*, 2001, *J Biol Chem* 276:16469-16477), indicating that the functional role of the N297 carbohydrate in mediating Fc/FcγR binding may be via the structural role it plays in determining the Fc conformation. This is supported by a collection of crystal structures of four different Fc glycoforms, which show that the composition of the oligosaccharide impacts the conformation of Cγ2 and as a result the Fc/FcγR interface (Krapp *et al.*, 2003, *J Mol Biol* 325:979-989).

[010] The features of antibodies discussed above - specificity for target, ability to mediate immune effector mechanisms, and long half-life in serum - make antibodies powerful therapeutics. Monoclonal antibodies are used therapeutically for the treatment of a variety of conditions including cancer, inflammation, and cardiovascular disease. There are currently over ten antibody products on the market and hundreds in development. In addition to antibodies, an antibody-like protein that is finding an expanding role in research and therapy is the Fc fusion (Chamow *et al.*, 1996, *Trends Biotechnol* 14:52-60; Ashkenazi *et al.*, 1997, *Curr Opin Immunol* 9:195-200). An Fc fusion is a protein wherein one or more polypeptides is operably linked to Fc. An Fc fusion combines the Fc region of an antibody, and thus its favorable effector functions and pharmacokinetics, with the target-binding region of a receptor, ligand, or some other protein or protein domain. The role of the latter is to mediate target recognition, and thus it is functionally analogous to the antibody variable region. Because of the structural and functional overlap of Fc fusions with antibodies, the discussion on antibodies in the present invention extends directly to Fc fusions.

[011] Despite such widespread use, antibodies are not optimized for clinical use. Two significant deficiencies of antibodies are their suboptimal anticancer potency and their demanding production requirements. These deficiencies are addressed by the present invention

[012] There are a number of possible mechanisms by which antibodies destroy tumor cells, including anti-proliferation via blockage of needed growth pathways, intracellular signaling leading to apoptosis, enhanced down regulation and/or turnover of receptors, CDC, ADCC, ADCP, and promotion of an adaptive immune response (Cragg *et al.*, 1999, *Curr Opin Immunol* 11:541-547; Glennie *et al.*, 2000, *Immunol Today* 21:403-410). Anti-tumor efficacy may be due to a combination of these mechanisms, and their relative importance in clinical therapy appears to be cancer dependent. Despite this arsenal of anti-tumor weapons, the potency of antibodies as anti-cancer agents is unsatisfactory, particularly given their high cost. Patient tumor response data show that monoclonal

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antibodies provide only a small improvement in therapeutic success over normal single-agent cytotoxic chemotherapeutics. For example, just half of all relapsed low-grade non-Hodgkin's lymphoma patients respond to the anti-CD20 antibody rituximab (McLaughlin *et al.*, 1998, *J Clin Oncol* 16:2825-2833). Of 166 clinical patients, 6% showed a complete response and 42% showed a partial response, with median response duration of approximately 12 months. Trastuzumab (Herceptin®, a registered trademark of Genentech), an anti-HER2/neu antibody for treatment of metastatic breast cancer, has less efficacy. The overall response rate using trastuzumab for the 222 patients tested was only 15%, with 8 complete and 26 partial responses and a median response duration and survival of 9 to 13 months (Cobleigh *et al.*, 1999, *J Clin Oncol* 17:2639-2648). Currently for anticancer therapy, any small improvement in mortality rate defines success. Thus there is a significant need to enhance the capacity of antibodies to destroy targeted cancer cells.

[013] A promising means for enhancing the anti-tumor potency of antibodies is via enhancement of their ability to mediate cytotoxic effector functions such as ADCC, ADCP, and CDC. The importance of FcγR-mediated effector functions for the anti-cancer activity of antibodies has been demonstrated in mice (Clynes *et al.*, 1998, *Proc Natl Acad Sci U S A* 95:652-656; Clynes *et al.*, 2000, *Nat Med* 6:443-446), and the affinity of interaction between Fc and certain FcγRs correlates with targeted cytotoxicity in cell-based assays (Shields *et al.*, 2001, *J Biol Chem* 276:6591-6604; Presta *et al.*, 2002, *Biochem Soc Trans* 30:487-490; Shields *et al.*, 2002, *J Biol Chem* 277:26733-26740). Additionally, a correlation has been observed between clinical efficacy in humans and their allotype of high (V158) or low (F158) affinity polymorphic forms of FcγRIIIa (Cartron *et al.*, 2002, *Blood* 99:754-758). Together these data suggest that an antibody with an Fc region optimized for binding to certain FcγRs may better mediate effector functions and thereby destroy cancer cells more effectively in patients. The balance between activating and inhibiting receptors is an important consideration, and optimal effector function may result from an Fc with enhanced affinity for activation receptors, for example FcγRI, FcγRIIa/c, and FcγRIIIa, yet reduced affinity for the inhibitory receptor FcγRIIb. Furthermore, because FcγRs can mediate antigen uptake and processing by antigen presenting cells, enhanced Fc/FcγR affinity may also improve the capacity of antibody therapeutics to elicit an adaptive immune response.

[014] Mutagenesis studies have been carried out on Fc towards various goals, with substitutions typically made to alanine (referred to as alanine scanning) or guided by sequence homology substitutions (Duncan *et al.*, 1988, *Nature* 332:563-564; Lund *et al.*, 1991, *J Immunol* 147:2657-2662; Lund *et al.*, 1992, *Mol Immunol* 29:53-59; Jefferis *et al.*, 1995, *Immunol Lett* 44:111-117; Lund *et al.*, 1995, *Faseb J* 9:115-119; Jefferis *et al.*, 1996, *Immunol Lett* 54:101-104; Lund *et al.*, 1996, *J Immunol* 157:4963-4969; Armour *et al.*, 1999, *Eur J Immunol* 29:2613-2624; Shields *et al.*, 2001, *J Biol Chem* 276:6591-6604; Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65) (US 5,624,821; US 5,885,573; PCT WO 00/42072; PCT WO 99/58572). The majority of substitutions reduce or ablate binding with FcγRs.

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However some success has been achieved at obtaining Fc variants with higher FcγR affinity. (See for example US 5,624,821, and PCT WO 00/42072). For example, Winter and colleagues substituted the human amino acid at position 235 of mouse IgG2b antibody (a glutamic acid to leucine mutation) that increased binding of the mouse antibody to human FcγRI by100-fold (Duncan *et al.*, 1988, *Nature* 332:563-564) (US 5,624,821). Shields *et al.* used alanine scanning mutagenesis to map Fc residues important to FcγR binding, followed by substitution of select residues with non-alanine mutations (Shields *et al.*, 2001, *J Biol Chem* 276:6591-6604; Presta *et al.*, 2002, *Biochem Soc Trans* 30:487-490) (PCT WO 00/42072). Several mutations disclosed in this study, including S298A, E333A, and K334A, show enhanced binding to the activating receptor FcγRIIIa and reduced binding to the inhibitory receptor FcγRIIb. These mutations were combined to obtain double and triple mutation variants that show additive improvements in binding. The best variant disclosed in this study is a S298A/E333A/K334A triple mutant with approximately a 1.7-fold increase in binding to F158 FcγRIIIa, a 5-fold decrease in binding to FcγRIIb, and a 2.1-fold enhancement in ADCC.

[015] Enhanced affinity of Fc for FcγR has also been achieved using engineered glycoforms generated by expression of antibodies in engineered or variant cell lines (Umaña *et al.*, 1999, *Nat Biotechnol* 17:176-180; Davies *et al.*, 2001, *Biotechnol Bioeng* 74:288-294; Shields *et al.*, 2002, *J Biol Chem* 277:26733-26740; Shinkawa *et al.*, 2003, *J Biol Chem* 278:3466-3473). This approach has generated substantial enhancements of the capacity of antibodies to bind FcγRIIIa and to mediate ADCC. Although there are practical limitations such as the growth efficiency of the expression strains under large scale production conditions, this approach for enhancing Fc/FcγR affinity and effector function is promising. Indeed, coupling of these alternate glycoform technologies with the Fc variants of the present invention may provide additive or synergistic effects for optimal effector function.

[016] Although there is a need for greater effector function, for some antibody therapeutics reduced or eliminated effector function may be desired. This is often the case for therapeutic antibodies whose mechanism of action involves blocking or antagonism but not killing of the cells bearing target antigen. In these cases depletion of target cells is undesirable and can be considered a side effect. For example, the ability of anti-CD4 antibodies to block CD4 receptors on T cells makes them effective anti-inflammatories, yet their ability to recruit FcγR receptors also directs immune attack against the target cells, resulting in T cell depletion (Reddy *et al.*, 2000, *J Immunol* 164:1925-1933). Effector function can also be a problem for radiolabeled antibodies, referred to as radioconjugates, and antibodies conjugated to toxins, referred to as immunotoxins. These drugs can be used to destroy cancer cells, but the recruitment of immune cells via Fc interaction with FcγRs brings healthy immune cells in proximity to the deadly payload (radiation or toxin), resulting in depletion of normal lymphoid tissue along with targeted cancer cells (Hutchins *et al.*, 1995, *Proc Natl Acad Sci U S A* 92:11980-11984; White *et al.*, 2001, *Annu Rev Med* 52:125-145). This problem can potentially be circumvented by using IgG isotypes that poorly recruit complement or effector cells, for example IgG2

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and IgG4. An alternate solution is to develop Fc variants that reduce or ablate binding (Alegre *et al.*, 1994, *Transplantation* 57:1537-1543; Hutchins *et al.*, 1995, *Proc Natl Acad Sci U S A* 92:11980-11984; Armour *et al.*, 1999, *Eur J Immunol* 29:2613-2624; Reddy *et al.*, 2000, *J Immunol* 164:1925-1933; Xu *et al.*, 2000, *Cell Immunol* 200:16-26; Shields *et al.*, 2001, *J Biol Chem* 276:6591-6604) (US 6,194,551; US 5,885,573; PCT WO 99/58572). A critical consideration for the reduction or elimination of effector function is that other important antibody properties not be perturbed. Fc variants should be engineered that not only ablate binding to FcγRs and/or C1q, but also maintain antibody stability, solubility, and structural integrity, as well as ability to interact with other important Fc ligands such as FcRn and proteins A and G.

[017] The present invention addresses another major shortcoming of antibodies, namely their demanding production requirements (Garber, 2001, *Nat Biotechnol* 19:184-185; Dove, 2002, *Nat Biotechnol* 20:777-779). Antibodies must be expressed in mammalian cells, and the currently marketed antibodies together with other high-demand biotherapeutics consume essentially all of the available manufacturing capacity. With hundreds of biologics in development, the majority of which are antibodies, there is an urgent need for more efficient and cheaper methods of production. The downstream effects of insufficient antibody manufacturing capacity are three-fold. First, it dramatically raises the cost of goods to the producer, a cost that is passed on to the patient. Second, it hinders industrial production of approved antibody products, limiting availability of high demand therapeutics to patients. Finally, because clinical trials require large amounts of a protein that is not yet profitable, the insufficient supply impedes progress of the growing antibody pipeline to market.

[018] Alternative production methods have been explored in attempts at alleviating this problem. Transgenic plants and animals are being pursued as potentially cheaper and higher capacity production systems (Chadd *et al.*, 2001, *Curr Opin Biotechnol* 12:188-194). Such expression systems, however, can generate glycosylation patterns significantly different from human glycoproteins. This may result in reduced or even lack of effector function because, as discussed above, the carbohydrate structure can significantly impact FcγR and complement binding. A potentially greater problem with nonhuman glycoforms may be immunogenicity; carbohydrates are a key source of antigenicity for the immune system, and the presence of nonhuman glycoforms has a significant chance of eliciting antibodies that neutralize the therapeutic, or worse cause adverse immune reactions. Thus the efficacy and safety of antibodies produced by transgenic plants and animals remains uncertain. Bacterial expression is another attractive solution to the antibody production problem. Expression in bacteria, for example *E. coli*, provides a cost-effective and high capacity method for producing proteins. For complex proteins such as antibodies there are a number of obstacles to bacterial expression, including folding and assembly of these complex molecules, proper disulfide formation, and solubility, stability, and functionality in the absence of glycosylation because proteins expressed in bacteria are not glycosylated. Full length unglycosylated antibodies that bind antigen have been successfully expressed in *E. coli* (Simmons *et al.*, 2002, *J Immunol*

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Methods 263:133-147), and thus, folding, assembly, and proper disulfide formation of bacterially expressed antibodies are possible in the absence of the eukaryotic chaperone machinery. However the ultimate utility of bacterially expressed antibodies as therapeutics remains hindered by the lack of glycosylation, which results in lack effector function and may result in poor stability and solubility. This will likely be more problematic for formulation at the high concentrations for the prolonged periods demanded by clinical use.

[019] An aglycosylated Fc with favorable solution properties and the capacity to mediate effector functions would be significantly enabling for the alternate production methods described above. By overcoming the structural and functional shortcomings of aglycosylated Fc, antibodies can be produced in bacteria and transgenic plants and animals with reduced risk of immunogenicity, and with effector function for clinical applications in which cytotoxicity is desired such as cancer. The present invention describes the utilization of protein engineering methods to develop stable, soluble Fc variants with effector function. Currently, such Fc variants do not exist in the art.

[020] In summary, there is a need for antibodies with enhanced therapeutic properties. Engineering of optimized or enhanced Fc variants is a promising approach to meeting this need. Yet a substantial obstacle to engineering Fc variants with the desired properties is the difficulty in predicting what amino acid modifications, out of the enormous number of possibilities, will achieve the desired goals, coupled with the inefficient production and screening methods for antibodies. Indeed one of the principle reasons for the incomplete success of the prior art is that approaches to Fc engineering have thus far involved hit-or-miss methods such as alanine scans or production of glycoforms using different expression strains. In these studies, the Fc modifications that were made were fully or partly random in hopes of obtaining variants with favorable properties. The present invention provides a variety of engineering methods, many of which are based on more sophisticated and efficient techniques, which may be used to overcome these obstacles in order to develop Fc variants that are optimized for the desired properties. The described engineering methods provide design strategies to guide Fc modification, computational screening methods to design favorable Fc variants, library generation approaches for determining promising variants for experimental investigation, and an array of experimental production and screening methods for determining the Fc variants with favorable properties.

SUMMARY OF THE INVENTION

[021] The present invention provides Fc variants that are optimized for a number of therapeutically relevant properties.

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[022] It is an object of the present invention to provide novel Fc positions at which amino acid modifications may be made to generate optimized Fc variants. Said Fc positions include 240, 244, 245, 247, 262, 263, 266, 299, 313, 325, 328, and 332, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. The present invention describes any amino acid modification at any of said novel Fc positions in order to generate an optimized Fc variant.

[023] It is a further object of the present invention to provide Fc variants that have been screened computationally. A computationally screened Fc variant is one that is predicted by the computational screening calculations described herein as having a significantly greater potential than random for being optimized for a desired property. In this way, computational screening serves as a prelude to or surrogate for experimental screening, and thus said computationally screened Fc variants are considered novel.

[024] It is a further object of the present invention to provide Fc variants that have been characterized using one or more of the experimental methods described herein. In one embodiment, said Fc variants comprise at least one amino acid substitution at a position selected from the group consisting of: 234, 235, 239, 240, 241, 243, 244, 245, 247, 262, 263, 264, 265, 266, 267, 269, 296, 297, 298, 299, 313, 325, 327, 328, 329, 330, and 332, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a preferred embodiment, said Fc variants comprise at least one substitution selected from the group consisting of L234D, L234E, L234N, L234Q, L234T, L234H, L234Y, L234I, L234V, L234F, L235D, L235S, L235N, L235Q, L235T, L235H, L235Y, L235I, L235V, L235F, S239D, S239E, S239N, S239Q, S239F, S239T, S239H, S239Y, V240I, V240A, V240T, V240M, F241W, F241L, F241Y, F241E, F241R, F243W, F243L F243Y, F243R, F243Q, P244H, P245A, P247V, P247G, V262I, V262A, V262T, V262E, V263I, V263A, V263T, V263M, V264L, V264I, V264W, V264T, V264R, V264F, V264M, V264Y, V264E, D265G, D265N, D265Q, D265Y, D265F, D265V, D265I, D265L, D265H, D265T, V266I, V266A, V266T, V266M, S267Q, S267L, E269H, E269Y, E269F, E269R, Y296E, Y296Q, Y296D, Y296N, Y296S, Y296T, Y296L, Y296I, Y296H, N297S, N297D, N297E, A298H, T299I, T299L, T299A, T299S, T299V, T299H, T299F, T299E, W313F, N325Q, N325L, N325I, N325D, N325E, N325A, N325T, N325V, N325H, A327N, A327L, L328M, L328D, L328E, L328N, L328Q, L328F, L328I, L328V, L328T, L328H, L328A, P329F, A330L, A330Y, A330V, A330I, A330F, A330R, A330H, I332D, I332E, I332N, I332Q, I332T, I332H, I332Y, and I332A, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a mostly preferred embodiment, said Fc variants are selected from the group consisting of V264L, V264I, F241W, F241L, F243W, F243L, F241L/F243L/V262I/V264I, F241W/F243W, F241W/F243W/V262A/V264A, F241L/V262I, F243L/V264I, F243L/V262I/V264W, F241Y/F243Y/V262T/V264T, F241E/F243R/V262E/V264R, F241E/F243Q/V262T/V264E, F241R/F243Q/V262T/V264R, F241E/F243Y/V262T/V264R, L328M, L328E, L328F, I332E, L328M/I332E, P244H, P245A, P247V, W313F, P244H/P245A/P247V, P247G, V264I/I332E, F241E/F243R/V262E/V264R/I332E, F241E/F243Q/V262T/V264E/I332E,

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F241R/F243Q/V262T/V264R/I332E, F241E/F243Y/V262T/V264R/I332E, S298A/I332E, S239E/I332E, S239Q/I332E, S239E, D265G, D265N, S239E/D265G, S239E/D265N, S239E/D265Q, Y296E, Y296Q, T299I, A327N, S267Q/A327S, S267L/A327S , A327L, P329F, A330L, A330Y, I332D, N297S, N297D, N297S/I332E, N297D/I332E, N297E/I332E, D265Y/N297D/I332E, D265Y/N297D/T299L/I332E, D265F/N297E/I332E, L328I/I332E, L328Q/I332E, I332N, I332Q, V264T, V264F, V240I, V263I, V266I, T299A, T299S, T299V, N325Q, N325L, N325I, S239D, S239N, S239F, S239D/I332D, S239D/I332E, S239D/I332N, S239D/I332Q, S239E/I332D, S239E/I332N, S239E/I332Q, S239N/I332D, S239N/I332E, S239N/I332N, S239N/I332Q, S239Q/I332D, S239Q/I332N, S239Q/I332Q, Y296D, Y296N, F241Y/F243Y/V262T/V264T/N297D/I332E, A330Y/I332E, V264I/A330Y/I332E, A330L/I332E, V264I/A330L/I332E, L234D, L234E, L234N, L234Q, L234T, L234H, L234Y, L234I, L234V, L234F, L235D, L235S, L235N, L235Q, L235T, L235H, L235Y, L235I, L235V, L235F, S239T, S239H, S239Y, V240A, V240T, V240M, V263A, V263T, V263M, V264M, V264Y, V266A, V266T, V266M, E269H, E269Y, E269F, E269R, Y296S, Y296T, Y296L, Y296I, A298H, T299H, A330V, A330I, A330F, A330R, A330H, N325D, N325E, N325A, N325T, N325V, N325H, L328D/I332E, L328E/I332E, L328N/I332E, L328Q/I332E, L328V/I332E, L328T/I332E, L328H/I332E, L328I/I332E, L328A, I332T, I332H, I332Y, I332A, S239E/V264I/I332E, S239Q/V264I/I332E, S239E/V264I/A330Y/I332E, S239E/V264I/S298A/A330Y/I332E, S239D/N297D/I332E, S239E/N297D/I332E, S239D/D265V/N297D/I332E, S239D/D265I/N297D/I332E, S239D/D265L/N297D/I332E, S239D/D265F/N297D/I332E, S239D/D265Y/N297D/I332E, S239D/D265H/N297D/I332E, S239D/D265T/N297D/I332E, V264E/N297D/I332E, Y296D/N297D/I332E, Y296E/N297D/I332E, Y296N/N297D/I332E, Y296Q/N297D/I332E, Y296H/N297D/I332E, Y296T/N297D/I332E, N297D/T299V/I332E, N297D/T299I/I332E, N297D/T299L/I332E, N297D/T299F/I332E, N297D/T299H/I332E, N297D/T299E/I332E, N297D/A330Y/I332E, N297D/S298A/A330Y/I332E, S239D/A330Y/I332E, S239N/A330Y/I332E, S239D/A330L/I332E, S239N/A330L/I332E, V264I/S298A/I332E, S239D/S298A/I332E, S239N/S298A/I332E, S239D/V264I/I332E, S239D/V264I/S298A/I332E, and S239D/V264I/A330L/I332E, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

[025] It is a further object of the present invention to provide an Fc variant that binds with greater affinity to one or more FcγRs. In one embodiment, said Fc variants have affinity for an FcγR that is more than 1-fold greater than that of the parent Fc polypeptide. In an alternate embodiment, said Fc variants have affinity for an FcγR that is more than 5-fold greater than that of the parent Fc polypeptide. In a preferred embodiment, said Fc variants have affinity for an FcγR that is between 5-fold and 300-fold greater than that of the parent Fc polypeptide. In one embodiment, said Fc variants comprise at least one amino acid substitution at a position selected from the group consisting of: 234, 235, 239, 240, 243, 264, 266, 328, 330, 332, and 325, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a preferred embodiment, said Fc variants comprise at least one amino acid substitution selected from the group consisting of: L234E, L234Y, L234I, L235D,

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L235S, L235Y, L235I, S239D, S239E, S239N, S239Q, S239T, V240I, V240M, F243L, V264I, V264T, V264Y, V266I, L328M, L328I, L328Q, L328D, L328V, L328T, A330Y, A330L, A330I, I332D, I332E, I332N, I332Q, and N325T, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a mostly preferred embodiment, said Fc variants are selected from the group consisting of V264I, F243L/V264I, L328M, I332E, L328M/I332E, V264I/I332E, S298A/I332E, S239E/I332E, S239Q/I332E, S239E, A330Y, I332D, L328I/I332E, L328Q/I332E, V264T, V240I, V266I, S239D, S239D/I332D, S239D/I332E, S239D/I332N, S239D/I332Q, S239E/I332D, S239E/I332N, S239E/I332Q, S239N/I332D, S239N/I332E, S239Q/I332D, A330Y/I332E, V264I/A330Y/I332E, A330L/I332E, V264I/A330L/I332E, L234E, L234Y, L234I, L235D, L235S, L235Y, L235I, S239T, V240M, V264Y, A330I, N325T, L328D/I332E, L328V/I332E, L328T/I332E, L328I/I332E, S239E/V264I/I332E, S239Q/V264I/I332E, S239E/V264I/A330Y/I332E, S239D/A330Y/I332E, S239N/A330Y/I332E, S239D/A330L/I332E, S239N/A330L/I332E, V264I/S298A/I332E, S239D/S298A/I332E, S239N/S298A/I332E, S239D/V264I/I332E, S239D/V264I/S298A/I332E, and S239D/V264I/A330L/I332E, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

[026] It is a further object of the present invention to provide Fc variantd that have a FcγRIIIa-fold:FcγRIIb-fold ratio greater than 1:1. In one embodiment, said Fc variants have a FcγRIIIa-fold:FcγRIIb-fold ratio greater than 11:1. In a preferred embodiment, said Fc variants have a FcγRIIIa-fold:FcγRIIb-fold ratio between 11:1 and 86:1. In one embodiment, said Fc variants comprise at least one amino acid substitution at a position selected from the group consisting of: 234, 235, 239, 240, 264, 296, 330, and I332, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a preferred embodiment, said Fc variants comprise at least one amino acid substitution selected from the group consisting of: L234Y, L234I, L235I, S239D, S239E, S239N, S239Q, V240A, V240M, V264I, V264Y, Y296Q, A330L, A330Y, A330I, I332D, and I332E, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a mostly preferred embodiment, said Fc variants are selected from the group consisting of: I332E, V264I/I332E, S239E/I332E, S239Q/I332E, Y296Q, A330L, A330Y, I332D, S239D, S239D/I332E, A330Y/I332E, V264I/A330Y/I332E, A330L/I332E, V264I/A330L/I332E, L234Y, L234I, L235I, V240A, V240M, V264Y, A330I, S239D/A330L/I332E, S239D/S298A/I332E, S239N/S298A/I332E, S239D/V264I/I332E, S239D/V264I/S298A/I332E, and S239D/V264I/A330L/I332E, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

[027] It is a further object of the present invention to provide Fc variants that mediate effector function more effectively in the presence of effector cells. In one embodiment, said Fc variants mediate ADCC that is greater than that mediated by the parent Fc polypeptide. In a preferred embodiment, said Fc variants mediate ADCC that is more than 5-fold greater than that mediated by the parent Fc polypeptide. In a mostly preferred embodiment, said Fc variants mediate ADCC that is between 5-fold and 50-fold greater than that mediated by the parent Fc polypeptide. In one

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embodiment, said Fc variants comprise at least one amino acid substitution at a position selected from the group consisting of: 234, 235, 239, 240, 243, 264, 266, 328, 330, 332, and 325, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a preferred embodiment, said Fc variants comprise at least one amino acid substitutions selected from the group consisting of: L234E, L234Y, L234I, L235D, L235S, L235Y, L235I, S239D, S239E, S239N, S239Q, S239T, V240I, V240M, F243L, V264I, V264T, V264Y, V266I, L328M, L328I, L328Q, L328D, L328V, L328T, A330Y, A330L, A330I, I332D, I332E, I332N, I332Q, and N325T, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a mostly preferred embodiment, said Fc variants are selected from the group consisting of: V264I, F243L/V264I, L328M, I332E, L328M/I332E, V264I/I332E, S298A/I332E, S239E/I332E, S239Q/I332E, S239E, A330Y, I332D, L328I/I332E, L328Q/I332E, V264T, V240I, V266I, S239D, S239D/I332D, S239D/I332E, S239D/I332N, S239D/I332Q, S239E/I332D, S239E/I332N, S239E/I332Q, S239N/I332D, S239N/I332E, S239Q/I332D, A330Y/I332E, V264I/A330Y/I332E, A330L/I332E, V264I/A330L/I332E, L234E, L234Y, L234I, L235D, L235S, L235Y, L235I, S239T, V240M, V264Y, A330I, N325T, L328D/I332E, L328V/I332E, L328T/I332E, L328I/I332E, S239E/V264I/I332E, S239Q/V264I/I332E, S239E/V264I/A330Y/I332E, S239D/A330Y/I332E, S239N/A330Y/I332E, S239D/A330L/I332E, S239N/A330L/I332E, V264I/S298A/I332E, S239D/S298A/I332E, S239N/S298A/I332E, S239D/V264I/I332E, S239D/V264I/S298A/I332E, and S239D/V264I/A330L/I332E, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

[028] It is a further object of the present invention to provide Fc variants that bind with weaker affinity to one or more FcγRs. In one embodiment, said Fc variants comprise at least one amino acid substitution at a position selected from the group consisting of: 234, 235, 239, 240, 241, 243, 244, 245, 247, 262, 263, 264, 265, 266, 267, 269, 296, 297, 298, 299, 313, 325, 327, 328, 329, 330, and 332, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a preferred embodiment, said Fc variants comprise an amino acid substitution at a position selected from the group consisting of: L234D, L234N, L234Q, L234T, L234H, L234V, L234F, L235N, L235Q, L235T, L235H, L235V, L235F, S239E, S239N, S239Q, S239F, S239H, S239Y, V240A, V240T, F241W, F241L, F241Y, F241E, F241R, F243W, F243L F243Y, F243R, F243Q, P244H, P245A, P247V, P247G, V262I, V262A, V262T, V262E, V263I, V263A, V263T, V263M, V264L, V264I, V264W, V264T, V264R, V264F, V264M, V264E, D265G, D265N, D265Q, D265Y, D265F, D265V, D265I, D265L, D265H, D265T, V266A, V266T, V266M, S267Q, S267L, E269H, E269Y, E269F, E269R, Y296E, Y296Q, Y296D, Y296N, Y296S, Y296T, Y296L, Y296I, Y296H, N297S, N297D, N297E, A298H, T299I, T299L, T299A, T299S, T299V, T299H, T299F, T299E, W313F, N325Q, N325L, N325I, N325D, N325E, N325A, N325V, N325H, A327N, A327L, L328M, 328E, L328N, L328Q, L328F, L328H, L328A, P329F, A330L, A330V, A330F, A330R, A330H, I332N, I332Q, I332T, I332H, I332Y, and I332A, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a mostly preferred embodiment, said Fc variants are selected from the group consisting of: V264L, F241W, F241L, F243W, F243L, F241L/F243L/V262I/V264I, F241W/F243W, F241W/F243W/V262A/V264A, F241L/V262I, F243L/V262I/V264W, F241Y/F243Y/V262T/V264T,

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F241E/F243R/V262E/V264R, F241E/F243Q/V262T/V264E, F241R/F243Q/V262T/V264R, F241E/F243Y/V262T/V264R, L328M, L328E, L328F, P244H, P245A, P247V, W313F, P244H/P245A/P247V, P247G, F241E/F243R/V262E/V264R/I332E, F241E/F243Y/V262T/V264R/I332E, D265G, D265N, S239E/D265G, S239E/D265N, S239E/D265Q, Y296E, Y296Q, T299I, A327N, S267Q/A327S, S267L/A327S , A327L, P329F, A330L, N297S, N297D, N297S/I332E, I332N, I332Q, V264F, V263I, T299A, T299S, T299V, N325Q, N325L, N325I, S239N, S239F, S239N/I332N, S239N/I332Q, S239Q/I332N, S239Q/I332Q, Y296D, Y296N, L234D, L234N, L234Q, L234T, L234H, L234V, L234F, L235N, L235Q, L235T, L235H, L235V, L235F, S239H, S239Y, V240A, V263T, V263M, V264M, V266A, V266T, V266M, E269H, E269Y, E269F, E269R, Y296S, Y296T, Y296L, Y296I, A298H, T299H, A330V, A330F, A330R, A330H, N325D, N325E, N325A, N325V, N325H, L328E/I332E, L328N/I332E, L328Q/I332E, L328H/I332E, L328A, I332T, I332H, I332Y, and I332A, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

[029] It is a further object of the present invention to provide Fc variants that mediate ADCC in the presence of effector cells less effectively. In one embodiment, said Fc variants comprise at least one amino acid substitution at a position selected from the group consisting of: 234, 235, 239, 240, 241, 243, 244, 245, 247, 262, 263, 264, 265, 266, 267, 269, 296, 297, 298, 299, 313, 325, 327, 328, 329, 330, and 332, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a preferred embodiment, said Fc variants comprise at least one amino acid substitution at a position selected from the group consisting of: L234D, L234N, L234Q, L234T, L234H, L234V, L234F, L235N, L235Q, L235T, L235H, L235V, L235F, S239E, S239N, S239Q, S239F, S239H, S239Y, V240A, V240T, F241W, F241L, F241Y, F241E, F241R, F243W, F243L F243Y, F243R, F243Q, P244H, P245A, P247V, P247G, V262I, V262A, V262T, V262E, V263I, V263A, V263T, V263M, V264L, V264I, V264W, V264T, V264R, V264F, V264M, V264E, D265G, D265N, D265Q, D265Y, D265F, D265V, D265I, D265L, D265H, D265T, V266A, V266T, V266M, S267Q, S267L, E269H, E269Y, E269F, E269R, Y296E, Y296Q, Y296D, Y296N, Y296S, Y296T, Y296L, Y296I, Y296H, N297S, N297D, N297E, A298H, T299I, T299L, T299A, T299S, T299V, T299H, T299F, T299E, W313F, N325Q, N325L, N325I, N325D, N325E, N325A, N325V, N325H, A327N, A327L, L328M, 328E, L328N, L328Q, L328F, L328H, L328A, P329F, A330L, A330V, A330F, A330R, A330H, I332N, I332Q, I332T, I332H, I332Y, and I332A, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a mostly preferred embodiment, said Fc variants are selected from the group consisting of: V264L, F241W, F241L, F243W, F243L, F241L/F243L/V262I/V264I, F241W/F243W, F241W/F243W/V262A/V264A, F241L/V262I, F243L/V262I/V264W, F241Y/F243Y/V262T/V264T, F241E/F243R/V262E/V264R, F241E/F243Q/V262T/V264E, F241R/F243Q/V262T/V264R, F241E/F243Y/V262T/V264R, L328M, L328E, L328F, P244H, P245A, P247V, W313F, P244H/P245A/P247V, P247G, F241E/F243R/V262E/V264R/I332E, F241E/F243Y/V262T/V264R/I332E, D265G, D265N, S239E/D265G, S239E/D265N, S239E/D265Q, Y296E, Y296Q, T299I, A327N, S267Q/A327S, S267L/A327S , A327L, P329F, A330L, N297S, N297D, N297S/I332E, I332N, I332Q, V264F, V263I, T299A, T299S, T299V, N325Q, N325L, N325I,

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S239N, S239F, S239N/I332N, S239N/I332Q, S239Q/I332N, S239Q/I332Q, Y296D, Y296N, L234D, L234N, L234Q, L234T, L234H, L234V, L234F, L235N, L235Q, L235T, L235H, L235V, L235F, S239H, S239Y, V240A, V263T, V263M, V264M, V266A, V266T, V266M, E269H, E269Y, E269F, E269R, Y296S, Y296T, Y296L, Y296I, A298H, T299H, A330V, A330F, A330R, A330H, N325D, N325E, N325A, N325V, N325H, L328E/I332E, L328N/I332E, L328Q/I332E, L328H/I332E, L328A, I332T, I332H, I332Y, and I332A, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

[030] It is a further object of the present invention to provide Fc variants that have improved function and/or solution properties as compared to the aglycosylated form of the parent Fc polypeptide. Improved functionality herein includes but is not limited to binding affinity to an Fc ligand. Improved solution properties herein includes but is not limited to stability and solubility. In one embodiment, said aglycosylated Fc variants bind to an FcγR with an affinity that is comparable to or better than the glycosylated parent Fc polypeptide. In an alternate embodiment, said Fc variants bind to an FcγR with an affinity that is within 0.4-fold of the glycosylated form of the parent Fc polypeptide. In one embodiment, said Fc variants comprise at least one amino acid substitution at a position selected from the group consisting of: 239, 241, 243, 262, 264, 265, 296, 297, 330, and 332, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a preferred embodiment, said Fc variants comprise an amino acid substitution selected from the group consisting of: S239D, S239E, F241Y, F243Y, V262T, V264T, V264E, D265Y, D265H, Y296N, N297D, A330Y, and I332E, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. In a mostly preferred embodiment, said Fc variants are selected from the group consisting of: N297D/I332E, F241Y/F243Y/V262T/V264T/N297D/I332E, S239D/N297D/I332E, S239E/N297D/I332E, S239D/D265Y/N297D/I332E, S239D/D265H/N297D/I332E, V264E/N297D/I332E, Y296N/N297D/I332E, and N297D/A330Y/I332E, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

[031] The present invention also provides methods for engineering optimized Fc variants. It is an object of the present invention to provide design strategies that may be used to guide Fc optimization. It is a further object of the present invention to provide computational screening methods that may be used to design Fc variants. It is a further object of the present invention to provide methods for generating libraries for experimental testing. It is a further object of the present invention to provide experimental production and screening methods for obtaining optimized Fc variants.

[032] The present invention provides isolated nucleic acids encoding the Fc variants described herein. The present invention provides vectors comprising said nucleic acids, optionally, operably linked to control sequences. The present invention provides host cells containing the vectors, and methods for producing and optionally recovering the Fc variants.

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[033] The present invention provides novel antibodies and Fc fusions that comprise the Fc variants disclosed herein. Said novel antibodies and Fc fusions may find use in a therapeutic product.

[034] The present invention provides compositions comprising antibodies and Fc fusions that comprise the Fc variants described herein, and a physiologically or pharmaceutically acceptable carrier or diluent.

[035] The present invention contemplates therapeutic and diagnostic uses for antibodies and Fc fusions that comprise the Fc variants disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[036] Figure 1. Antibody structure and function. Shown is a model of a full length human IgG1 antibody, modeled using a humanized Fab structure from pdb accession code 1CE1 (James *et al.*, 1999, *J Mol Biol* 289:293-301) and a human IgG1 Fc structure from pdb accession code 1DN2 (DeLano *et al.*, 2000, *Science* 287:1279-1283). The flexible hinge that links the Fab and Fc regions is not shown. IgG1 is a homodimer of heterodimers, made up of two light chains and two heavy chains. The Ig domains that comprise the antibody are labeled, and include V_L and C_L for the light chain, and V_H, Cgamma1 (Cγ1), Cgamma2 (Cγ2), and Cgamma3 (Cγ3) for the heavy chain. The Fc region is labeled. Binding sites for relevant proteins are labeled, including the antigen binding site in the variable region, and the binding sites for FcγRs, FcRn, C1q, and proteins A and G in the Fc region.

[037] Figure 2. The Fc/FcγRIIIb complex structure 1IIS. Fc is shown as a gray ribbon diagram, and FcγRIIIb is shown as a black ribbon. The N297 carbohydrate is shown as black sticks.

[038] Figure 3. The amino acid sequence of the heavy chain of the antibody alemtuzumab (Campath®, a registered trademark of Ilex Pharmaceuticals LP), illustrating positions numbered sequentially (2 lines above the amino acid sequence) and positions numbered according to the EU index as in Kabat (2 lines below the amino acid sequence. The approximate beginnings of Ig domains VH1, Cγ1, the hinge, Cγ2, and Cγ3 are also labeled above the sequential numbering. Polymorphisms have been observed at a number of Fc positions, including but not limited to Kabat 270, 272, 312, 315, 356, and 358, and thus slight differences between the presented sequence and sequences in the prior art may exist.

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[039] Figure 4. Experimental library residues mapped onto the Fc/FcγRIIIb complex structure 1IIS. Fc is shown as a gray ribbon diagram, and FcγRIIIb is shown as a black ribbon. Experimental library residues are shown as black ball and sticks. The N297 carbohydrate is shown as black sticks.

[040] Figure 5. The human IgG1 Fc sequence showing positions relevant to the design of the Fc variant experimental library. The sequence includes the hinge region, domain Cγ2, and domain Cγ3. Residue numbers are according to the EU index as in Kabat. Positions relevant to the experimental library are underlined. Because of observed polymorphic mutations at a number of Fc positions, slight differences between the presented sequence and sequences in the literature may exist.

[041] Figure 6. Expression of Fc variant and wild type (WT) proteins of alemtuzumab in 293T cells. Plasmids containing alemtuzumab heavy chain genes (WT or variants) were co-transfected with plasmid containing the alemtuzumab light chain gene. Media were harvested 5 days after transfection. For each transfected sample, 10ul medium was loaded on a SDS-PAGE gel for Western analysis. The probe for Western was peroxidase-conjugated goat-anti human IgG (Jackson Immuno-Research, catalog # 109-035-088). WT: wild type alemtuzumab; 1-10: alemtuzumab variants. H and L indicate antibody heavy chain and light chain, respectively.

[042] Figure 7. Purification of alemtuzumab using protein A chromatography. WT alemtuzumab proteins was expressed in 293T cells and the media was harvested 5 days after transfection. The media were diluted 1:1 with PBS and purified with protein A (Pierce, Catalog # 20334). O: original sample before purification; FT: flow through; E: elution; C: concentrated final sample. The left picture shows a Simple Blue-stained SDS-PAGE gel, and the right shows a western blot labeled using peroxidase-conjugated goat-anti human IgG.

[043] Figure 8. Production of deglycosylated antibodies. Wild type and variants of alemtuzumab were expressed in 293T cells and purified with protein A chromatography. Antibodies were incubated with peptide-N-glycosidase (PNGase F) at 37°C for 24h. For each antibody, a mock treated sample (-PNGase F) was done in parallel. WT: wild-type alemtuzumab; #15, #16, #17, #18, #22: alemtuzumab variants F241E/F243R/V262E/V264R, F241E/F243Q/V262T/V264E, F241R/F243Q/V262T/V264R, F241E/F243Y/V262T/V264R, and I332E respectively. The faster migration of the PNGase F treated versus the mock treated samples represents the deglycosylated heavy chains.

[044] Figure 9. Alemtuzumab expressed from 293T cells binds its antigen. The antigenic CD52 peptide, fused to GST, was expressed in *E. coli* BL21 (DE3) under IPTG induction. Both uninduced and induced samples were run on a SDS-PAGE gel, and transferred to PVDF membrane. For western analysis, either alemtuzumab from Sotec (□-CD52, Sotec) (final concentration 2.5ng/ul) or

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media of transfected 293T cells (Campath, Xencor) (final alemtuzumab concentration approximately 0.1-0.2ng/ul) were used as primary antibody, and peroxidase-conjugated goat-anti human IgG was used as secondary antibody. M: pre-stained marker; U: un-induced sample for GST-CD52; I: induced sample for GST-CD52.

[045] Figure 10. Expression and purification of extracellular region of human V158 FcγRIIIa. Tagged FcγRIIIa was transfected in 293T cells, and media containing secreted FcγRIIIa were harvested 3 days later and purified using affinity chromatography. 1: media; 2: flow through; 3: wash; 4-8: serial elutions. Both simple blue-stained SDS-PAGE gel and western result are shown. For the western blot, membrane was probed with anti-GST antibody.

[046] Figure 11. Binding to human V158 FcγRIIIa by select alemtuzumab Fc variants from the experimental library as determined by the AlphaScreen™ assay, described in Example 2. In the presence of competitor antibody (Fc variant or WT alemtuzumab) a characteristic inhibition curve is observed as a decrease in luminescence signal. Phosphate buffer saline (PBS) alone was used as the negative control. These data were normalized to the maximum and minimum luminescence signal provided by the baselines at low and high concentrations of competitor antibody respectively. The curves represent the fits of the data to a one site competition model using nonlinear regression. These fits provide IC50s for each antibody, illustrated for WT and S239D by the dotted lines.

[047] Figures 12. AlphaScreen™ assay showing binding of select alemtuzumab Fc variants to human FcγRIIb. The data were normalized, and the curves represent the fits of the data to a one site competition model. PBS was used as a negative control.

[048] Figures 13. AlphaScreen™ assay showing binding of select alemtuzumab Fc variants to human Val158 FcγRIIIa. The data were normalized, and the curves represent the fits of the data to a one site competition model. PBS was used as a negative control.

[049] Figure 14. AlphaScreen™ assay measuring binding to human V158 FcγRIIIa by select Fc variants in the context of rituximab. The data were normalized, and the curves represent the fits of the data to a one site competition model. PBS was used as a negative control.

[050] Figure 15. AlphaScreen™ assay measuring binding to human V158 FcγRIIIa by select Fc variants in the context of trastuzumab. The data were normalized, and the curves represent the fits of the data to a one site competition model. PBS was used as a negative control.

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[051] Figures 16a – 16b. AlphaScreen™ assay comparing binding of select alemtuzumab Fc variants to human V158 FcγRIIIa (Figure 16a) and human FcγRIIb (Figure 16b). The data were normalized, and the curves represent the fits of the data to a one site competition model. PBS was used as a negative control.

[052] Figure 17. AlphaScreen™ assay measuring binding to human V158 FcγRIIIa by select Fc variants in the context of trastuzumab. The data were normalized, and the curves represent the fits of the data to a one site competition model.

[053] Figures 18. AlphaScreen™ assay showing binding of select alemtuzumab Fc variants to human R131 FcγRIIa. The data were normalized, and the curves represent the fits of the data to a one site competition model.

[054] Figures 19a and 19b. AlphaScreen™ assay showing binding of select alemtuzumab Fc variants to human V158 FcγRIIIa. The data were normalized, and the curves represent the fits of the data to a one site competition model. PBS was used as a negative control.

[055] Figure 20. AlphaScreen™ assay showing binding of aglycosylated alemtuzumab Fc variants to human V158 FcγRIIIa. The data were normalized, and the curves represent the fits of the data to a one site competition model. PBS was used as a negative control.

[056] Figure 21. AlphaScreen™ assay comparing human V158 FcγRIIIa binding by select alemtuzumab Fc variants in glycosylated (solid symbols, solid lines) and deglycosylated (open symbols, dotted lines). The data were normalized, and the curves represent the fits of the data to a one site competition model.

[057] Figures 22a – 22b. AlphaScreen™ assay showing binding of select alemtuzumab Fc variants to the V158 (Figure 22a) and F158 (Figure 22b) allotypes of human FcγRIIIa. The data were normalized, and the curves represent the fits of the data to a one site competition model. PBS was used as a negative control.

[058] Figures 23a – 23d. Figures 23a and 23b show the correlation between SPR Kd's and AlphaScreen™ IC50's from binding of select alemtuzumab Fc variants to V158 FcγRIIIa (Figure 23a)

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and F158 FcγRIIIa (Figure 23b). Figures 23c and 23d show the correlation between SPR and AlphaScreen™ fold-improvements over WT for binding of select alemtuzumab Fc variants to V158 FcγRIIIa (Figure 23c) and F158 FcγRIIIa (Figure 23d). Binding data are presented in Table 62. The lines through the data represent the linear fits of the data, and the r^2 values indicate the significance of these fits.

[059] Figures 24a – 24b. Cell-based ADCC assays of select Fc variants in the context of alemtuzumab. ADCC was measured using the DELFIA® EuTDA-based cytotoxicity assay (Perkin Elmer, MA), as described in Example 7, using DoHH-2 lymphoma target cells and 50-fold excess human PBMCs. Figure 24a is a bar graph showing the raw fluorescence data for the indicated alemtuzumab antibodies at 10 ng/ml. The PBMC bar indicates basal levels of cytotoxicity in the absence of antibody. Figure 24b shows the dose-dependence of ADCC on antibody concentration for the indicated alemtuzumab antibodies, normalized to the minimum and maximum fluorescence signal provided by the baselines at low and high concentrations of antibody respectively. The curves represent the fits of the data to a sigmoidal dose-response model using nonlinear regression.

[060] Figures 25a – 25b. Cell-based ADCC assays of select Fc variants in the context of rituximab. ADCC was measured using the DELFIA® EuTDA-based cytotoxicity assay, as described in Example 7, using WIL2-S lymphoma target cells and 50-fold excess human PBMCs. Figure 25a is a bar graph showing the raw fluorescence data for the indicated rituximab antibodies at 1 ng/ml. The PBMC bar indicates basal levels of cytotoxicity in the absence of antibody. Figure 25b shows the dose-dependence of ADCC on antibody concentration for the indicated rituximab antibodies, normalized to the minimum and maximum fluorescence signal provided by the baselines at low and high concentrations of antibody respectively. The curves represent the fits of the data to a sigmoidal dose-response model using nonlinear regression.

[061] Figures 26a – 26c. Cell-based ADCC assays of select Fc variants in the context of trastuzumab. ADCC was measured using the DELFIA® EuTDA-based cytotoxicity assay, as described in Example 7, using BT474 and Sk-Br-3 breast carcinoma target cells and 50-fold excess human PBMCs. Figure 26a is a bar graph showing the raw fluorescence data for the indicated trastuzumab antibodies at 1 ng/ml. The PBMC bar indicates basal levels of cytotoxicity in the absence of antibody. Figures 26b and 26c show the dose-dependence of ADCC on antibody concentration for the indicated trastuzumab antibodies, normalized to the minimum and maximum fluorescence signal provided by the baselines at low and high concentrations of antibody respectively. The curves represent the fits of the data to a sigmoidal dose-response model using nonlinear regression.

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[062] Figures 27a – 27b. Capacity of select Fc variants to mediate binding and activation of complement. Figure 27a shows an AlphaScreen™ assay measuring binding of select alemtuzumab Fc variants to C1q. The data were normalized to the maximum and minimum luminescence signal provided by the baselines at low and high concentrations of competitor antibody respectively. The curves represent the fits of the data to a one site competition model using nonlinear regression. Figure 27b shows a cell-based assay measuring capacity of select rituximab Fc variants to mediate CDC. CDC assays were performed using Amar Blue to monitor lysis of Fc variant and WT rituximab - opsonized WIL2-S lymphoma cells by human serum complement (Quidel, San Diego, CA). The dose-dependence on antibody concentration of complement-mediated lysis is shown for the indicated rituximab antibodies, normalized to the minimum and maximum fluorescence signal provided by the baselines at low and high concentrations of antibody respectively. The curves represent the fits of the data to a sigmoidal dose-response model using nonlinear regression.

[063] Figure 28. AlphaScreen™ assay measuring binding of select alemtuzumab Fc variants to bacterial protein A, as described in Example 9. The data were normalized, and the curves represent the fits of the data to a one site competition model. PBS was used as a negative control.

[064] Figure 29. AlphaScreen™ assay measuring binding of select alemtuzumab Fc variants to mouse FcγRIII, as described in Example 10. The data were normalized, and the curves represent the fits of the data to a one site competition model. PBS was used as a negative control.

[065] Figure 30. AlphaScreen™ assay measuring binding to human V158 FcγRIIIa by select trastuzumab Fc variants expressed in 293T and CHO cells, as described in Example 11. The data were normalized, and the curves represent the fits of the data to a one site competition model. PBS was used as a negative control.

[066] Figures 31a – 31c. Sequences showing improved anti-CD20 antibodies. The light and heavy chain sequences of rituximab are presented in Figure 31a and Figure 31b respectively, and are taken from translated Sequence 3 of US 5,736,137. Relevant positions in Figure 31b are bolded, including S239, V240, V264I, N297, S298, A330, and I332. Figure 31c shows the improved anti-CD20 antibody heavy chain sequences, with variable positions designated in bold as X_1, X_2, X_3, X_4, X_5, X_6, and Z_1. The table below the sequence provides possible substitutions for these positions. The improved anti-CD20 antibody sequences comprise at least one non-WT amino acid selected from the group of possible substitutions for X_1, X_2, X_3, X_4, X_5, and X_6. These improved anti-CD20 antibody sequences may also comprise a substitution Z_1. These positions are numbered according to the EU index as in Kabat, and thus do not correspond to the sequential order in the sequence.

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DETAILED DESCRIPTION OF THE INVENTION

[067] In order that the invention may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[068] By "ADCC" or "antibody dependent cell-mediated cytotoxicity" as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell.

[069] By "ADCP" or antibody dependent cell-mediated phagocytosis as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

[070] By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. The preferred amino acid modification herein is a substitution. **TT

[071] By "antibody" herein is meant a protein consisting of one or more polypeptides substantially encoded by all or part of the recognized immunoglobulin genes. The recognized immunoglobulin genes, for example in humans, include the kappa (□), lambda (□), and heavy chain genetic loci, which together comprise the myriad variable region genes, and the constant region genes mu (□), delta (□), gamma (γ), sigma (□), and alpha (□) which encode the IgM, IgD, IgG, IgE, and IgA isotypes respectively. Antibody herein is meant to include full length antibodies and antibody fragments, and may refer to a natural antibody from any organism, an engineered antibody, or an antibody generated recombinantly for experimental, therapeutic, or other purposes as further defined below. Thus, "antibody" includes both polyclonal and monoclonal antibody (mAb). Methods of preparation and purification of monoclonal and polyclonal antibodies are known in the art and e.g., are described in Harlow and Lane, Antibodies: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1988). As outlined herein, "antibody" specifically includes Fc variants described herein, "full length" antibodies including the Fc variant fragments described herein, and Fc variant fusions to other proteins as described herein.

[072] n some embodiments, antibodies can be neutralizing or inhibitory, or stimulatory, and in preferred embodiments, as described herein, the stimulatory activity is measured by an increase in affinitiy of a variant antibody to a receptor, as compared to either the parent antibody (e.g. when a non-naturally occurring variant is used as the starting point for the computation analysis herein), or to the original wild-type antibody. Accordingly, by "neutralization," "neutralize," "neutralizing" and grammatical equivalents herein is meant to inhibit or lessen the biological effect of the antibody, in some cases by binding (e.g. competitively) to a antigen and avoiding or decreasing the biological effect of binding, or by binding that results in decreasing the biological effect of binding.

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[073] The term "antibody" include antibody fragments, as are known in the art, such as Fab, Fab', F(ab')2, Fcs or other antigen-binding subsequences of antibodies, such as, single chain antibodies (Fv for example), chimeric antibodies, etc., either produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. Particularly preferred are Fc variants as described herein. The term "antibody" further comprises polyclonal antibodies and mAbs which can be agonist or antagonist antibodies.

[074] The antibodies of the invention specifically bind to Fc receptors, as outlined herein. By "specifically bind" herein is meant that the LC antibodies have a binding constant in the range of at least 10^{-4} - 10^{-6} M^{-1}, with a preferred range being 10^{-7} - 10^{-9} M^{-1}.
In a preferred embodiment, the antibodies of the invention are humanized. Using current monoclonal antibody technology one can produce a humanized antibody to virtually any target antigen that can be identified [Stein, Trends Biotechnol. 15:88-90 (1997)]. Humanized forms of non-human (e.g., murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fc, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [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)]. Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., supra; Riechmann et al., supra; and Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Additional examples of humanized murine monoclonal antibodies are also known in the art, e.g., antibodies binding human protein C [O'Connor et al., Protein Eng. 11:321-8 (1998)], interleukin 2 receptor [Queen et al., Proc. Natl. Acad. Sci., U.S.A. 86:10029-33 (1989)], and human epidermal growth factor receptor 2 [Carter et al., Proc. Natl. Acad. Sci. U.S.A. 89:4285-9 (1992)]. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has

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

[075] In a preferred embodiment, the antibodies of the invention are based on human sequences, and are thus human sequences are used as the "base" sequences, against which other sequences, such as rat, mouse and monkey sequences. In order to establish homology to primary sequence or structure, the amino acid sequence of a precursor or parent Fc is directly compared to the human Fc sequence outlined herein. After aligning the sequences, using one or more of the homology alignment programs described herein (for example using conserved residues as between species), allowing for necessary insertions and deletions in order to maintain alignment (i.e., avoiding the elimination of conserved residues through arbitrary deletion and insertion), the residues equivalent to particular amino acids in the primary sequence of human Fc are defined. Alignment of conserved residues preferably should conserve 100% of such residues. However, alignment of greater than 75% or as little as 50% of conserved residues is also adequate to define equivalent residues (sometimes referred to herein as "corresponding residues").

[076] Equivalent residues may also be defined by determining homology at the level of tertiary structure for an Fc fragment whose tertiary structure has been determined by x-ray crystallography. Equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the parent or precursor (N on N, CA on CA, C on C and O on O) are within 0.13 nm and preferably 0.1 nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the Fc variant fragment.

[077] Specifically included within the definition of "antibody" are aglycosylated antibodies. By "aglycosylated antibody" as used herein is meant an antibody that lacks carbohydrate attached at position 297 of the Fc region, wherein numbering is according to the EU system as in Kabat. The aglycosylated antibody may be a deglycosylated antibody, that is an antibody for which the Fc carbohydrate has been removed, for example chemically or enzymatically. Alternatively, the aglycosylated antibody may be a nonglycosylated or unglycosylated antibody, that is an antibody that was expressed without Fc carbohydrate, for example by mutation of one or residues that encode the glycosylation pattern or by expression in an organism that does not attach carbohydrates to proteins, for example bacteria.

[078] Specifically included within the definition of "antibody" are full-length antibodies that contain an Fc variant portion. .By "full length antibody" herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions. For example, in most mammals, including humans and mice, the full length antibody of the IgG class is a tetramer and consists of two identical pairs of two immunoglobulin chains, each pair having one light and one heavy

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chain, each light chain comprising immunoglobulin domains V_L and C_L, and each heavy chain comprising immunoglobulin domains V_H, $C\gamma1$, $C\gamma2$, and $C\gamma3$. In some mammals, for example in camels and llamas, IgG antibodies may consist of only two heavy chains, each heavy chain comprising a variable domain attached to the Fc region. By "IgG" as used herein is meant a polypeptide belonging to the class of antibodies that are substantially encoded by a recognized immunoglobulin gamma gene. In humans this class comprises IgG1, IgG2, IgG3, and IgG4. In mice this class comprises IgG1, IgG2a, IgG2b, IgG3.

[079] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids or any non-natural analogues that may be present at a specific, defined position. By "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures, i.e. "analogs", such as peptoids (see Simon et al., PNAS USA 89(20):9367 (1992)) particularly when LC peptides are to be administered to a patient. Thus "amino acid", or "peptide residue", as used herein means both naturally occurring and synthetic amino acids. For example, homophenylalanine, citrulline and noreleucine are considered amino acids for the purposes of the invention. "Amino acid" also includes imino acid residues such as proline and hydroxyproline. The side chain may be in either the (R) or the (S) configuration. In the preferred embodiment, the amino acids are in the (S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard in vivo degradation.

[080] By "computational screening method" herein is meant any method for designing one or more mutations in a protein, wherein said method utilizes a computer to evaluate the energies of the interactions of potential amino acid side chain substitutions with each other and/or with the rest of the protein. As will be appreciated by those skilled in the art, evaluation of energies, referred to as energy calculation, refers to some method of scoring one or more amino acid modifications. Said method may involve a physical or chemical energy term, or may involve knowledge-, statistical-, sequence-based energy terms, and the like. The calculations that compose a computational screening method are herein referred to as "computational screening calculations".

[081] By "effector function" as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to ADCC, ADCP, and CDC. By "effector cell" as used herein is meant a cell of the immune system that expresses one or more Fc receptors and mediates one or more effector functions. Effector cells include but are not limited to monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, and $\gamma\gamma$ T cells, and may be from any organism including but not limited to humans, mice,

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rats, rabbits, and monkeys. By "library" herein is meant a set of Fc variants in any form, including but not limited to a list of nucleic acid or amino acid sequences, a list of nucleic acid or amino acid substitutions at variable positions, a physical library comprising nucleic acids that encode the library sequences, or a physical library comprising the Fc variant proteins, either in purified or unpurified form.

[082] By "Fc", "Fc region", FC polypeptide", etc. as used herein is meant an antibody as defined herein that includes the polypeptides comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM Fc may include the J chain. For IgG, as illustrated in Figure 1, Fc comprises immunoglobulin domains Cgamma2 and Cgamma3 (Cγ2 and Cγ3) and the hinge between Cgamma1 (Cγ1) and Cgamma2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. Fc may refer to this region in isolation, or this region in the context of an antibody, antibody fragment, or Fc fusion. An Fc may be an antibody, Fc fusion, or an protein or protein domain that comprises Fc. Particularly preferred are Fc variants, which are non-naturally occurring variants of an Fc.

[083] By "Fc fusion" as used herein is meant a protein wherein one or more polypeptides is operably linked to Fc. Fc fusion is herein meant to be synonymous with the terms "immunoadhesin", "Ig fusion", "Ig chimera", and "receptor globulin" (sometimes with dashes) as used in the prior art (Chamow *et al.*, 1996, *Trends Biotechnol* 14:52-60; Ashkenazi *et al.*, 1997, *Curr Opin Immunol* 9:195-200). An Fc fusion combines the Fc region of an immunoglobulin with a fusion partner, which in general can be any protein, including, but not limited to, the target-binding region of a receptor, an adhesion molecule, a ligand, an enzyme, or some other protein or protein domain. The role of the non-Fc part of an Fc fusion is to mediate target binding, and thus it is functionally analogous to the variable regions of an antibody.

[084] By "Fc gamma receptor" or "FcγR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and are substantially encoded by the FcγR genes. In humans this family includes but is not limited to FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2) (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. An FcγR may be from any organism, including but not limited to humans,

mice, rats, rabbits, and monkeys. Mouse FcγRs include but are not limited to FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes.

[085] By "Fc ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an antibody to form an Fc-ligand complex. Fc ligands include but are not limited to FcγRs, FcγRs, FcγRs, FcRn, C1q, C3, mannan binding lectin, mannose receptor, *staphylococcal* protein A, *streptococcal* protein G, and viral FcγR. Fc ligands may include undiscovered molecules that bind Fc

[086] By "IgG" as used herein is meant a polypeptide belonging to the class of antibodies that are substantially encoded by a recognized immunoglobulin gamma gene. In humans this class comprises IgG1, IgG2, IgG3, and IgG4. In mice this class comprises IgG1, IgG2a, IgG2b, IgG3. By "immunoglobulin (Ig)" herein is meant a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. Immunoglobulins include but are not limited to antibodies. Immunoglobulins may have a number of structural forms, including but not limited to full length antibodies, antibody fragments, and individual immunoglobulin domains. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin that exists as a distinct structural entity as ascertained by one skilled in the art of protein structure. Ig domains typically have a characteristic □-sandwich folding topology. The known Ig domains in the IgG class of antibodies are V_H, Cγ1, Cγ2, Cγ3, V_L, and C_L.

[087] By "parent polypeptide" or "precursor polypeptide" (including Fc parent or precursors) as used herein is meant a polypeptide that is subsequently modified to generate a variant. Said parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it. Accordingly, by "parent Fc polypeptide" as used herein is meant an unmodified Fc polypeptide that is modified to generate a variant, and by "parent antibody" as used herein is meant an unmodified antibody that is modified to generate a variant antibody.

[088] As outlined above, certain positions of the Fc molecule can be altered. By "position" as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example the EU index as in Kabat. For example, position 297 is a position in the human antibody IgG1. Corresponding positions are determined as outlined above, generally through alignment with other parent sequences.

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[089] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Daragine 297 (also referred to as N297, also referred to as N297) is a residue in the human antibody IgG1.

[090] By "target antigen" as used herein is meant the molecule that is bound specifically by the variable region of a given antibody. A target antigen may be a protein, carbohydrate, lipid, or other chemical compound.

[091] By "target cell" as used herein is meant a cell that expresses a target antigen.

[092] By "variable region" as used herein is meant the region of an immunoglobulin that comprises one or more Ig domains substantially encoded by any of the V□, V□, and/or V_H genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

[093] By "variant polypeptide" as used herein is meant a polypeptide sequence that differs from that of a parent polypeptide sequence by virtue of at least one amino acid modification. Variant polypeptide may refer to the polypeptide itself, a composition comprising the polypeptide, or the amino sequence that encodes it. Preferably, the variant polypeptide has at least one amino acid modification compared to the parent polypeptide, e.g. from about one to about ten amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. The variant polypeptide sequence herein will preferably possess at least about 80% homology with a parent polypeptide sequence, and most preferably at least about 90% homology, more preferably at least about 95% homology. Accordingly, by "Fc variant" as used herein is meant an Fc sequence that differs from that of a parent Fc sequence by virtue of at least one amino acid modification. An Fc variant may only encompass an Fc region, or may exist in the context of an antibody, Fc fusion, or other polypeptide that is substantially encoded by Fc. Fc variant may refer to the Fc polypeptide itself, compositions comprising the Fc variant polypeptide, or the amino acid sequence that encodes it.

[094] For all positions discussed in the present invention, numbering of an immunoglobulin heavy chain is according to the EU index (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Svice, National Institutes of Health, Bethesda). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

[095] The Fc variants of the present invention may be optimized for a variety of properties. Properties that may be optimized include but are not limited to enhanced or reduced affinity for an

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FcγR. In a preferred embodiment, the Fc variants of the present invention are optimized to possess enhanced affinity for a human activating FcγR, preferably FcγRI, FcγRIIa, FcγRIIc, FcγRIIIa, and FcγRIIIb, most preferably FcγRIIIa. In an alternately preferred embodiment, the Fc variants are optimized to possess reduced affinity for the human inhibitory receptor FcγRIIb. These preferred embodiments are anticipated to provide antibodies and Fc fusions with enhanced therapeutic properties in humans, for example enhanced effector function and greater anti-cancer potency. In an alternate embodiment, the Fc variants of the present invention are optimized to have reduced or ablated affinity for a human FcγR, including but not limited to FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, and FcγRIIIb. These embodiments are anticipated to provide antibodies and Fc fusions with enhanced therapeutic properties in humans, for example reduced effector function and reduced toxicity. Preferred embodiments comprise optimization of Fc binding to a human FcγR, however in alternate embodiments the Fc variants of the present invention possess enhanced or reduced affinity for FcγRs from nonhuman organisms, including but not limited to mice, rats, rabbits, and monkeys. Fc variants that are optimized for binding to a nonhuman FcγR may find use in experimentation. For example, mouse models are available for a variety of diseases that enable testing of properties such as efficacy, toxicity, and pharmacokinetics for a given drug candidate. As is known in the art, cancer cells can be grafted or injected into mice to mimic a human cancer, a process referred to as xenografting. Testing of antibodies or Fc fusions that comprise Fc variants that are optimized for one or more mouse FcγRs, may provide valuable information with regard to the efficacy of the antibody or Fc fusion, its mechanism of action, and the like. The Fc variants of the present invention may also be optimized for enhanced functionality and/or solution properties in aglycosylated form. In a preferred embodiment, the aglycosylated Fc variants of the present invention bind an Fc ligand with greater affinity than the aglycosylated form of the parent Fc polypeptide. Said Fc ligands include but are not limited to FcγRs, C1q, FcRn, and proteins A and G, and may be from any source including but not limited to human, mouse, rat, rabbit, or monkey, preferably human. In an alternately preferred embodiment, the Fc variants are optimized to be more stable and/or more soluble than the aglycosylated form of the parent Fc polypeptide. An Fc variant that is engineered or predicted to display any of the aforementioned optimized properties is herein referred to as an "optimized Fc variant".

[096] The Fc variants of the present invention may be derived from parent Fc polypeptides that are themselves from a wide range of sources. The parent Fc polypeptide may be substantially encoded by one or more Fc genes from any organism, including but not limited to humans, mice, rats, rabbits, camels, llamas, dromedaries, monkeys, preferably mammals and most preferably humans and mice. In a preferred embodiment, the parent Fc polypeptide composes an antibody, referred to as the parent antibody. The parent antibody may be fully human, obtained for example using transgenic mice (Bruggemann *et al.*, 1997, *Curr Opin Biotechnol* 8:455-458) or human antibody libraries coupled with selection methods (Griffiths *et al.*, 1998, *Curr Opin Biotechnol* 9:102-108). The parent antibody need not be naturally occurring. For example, the parent antibody may be an engineered antibody,

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including but not limited to chimeric antibodies and humanized antibodies (Clark, 2000, *Immunol Today* 21:397-402). The parent antibody may be an engineered variant of an antibody that is substantially encoded by one or more natural antibody genes. In one embodiment, the parent antibody has been affinity matured, as is known in the art. Alternatively, the antibody has been modified in some other way, for example as described in USSN 10/339788, filed on March 3, 2003.

[097] The Fc variants of the present invention may be substantially encoded by immunoglobulin genes belonging to any of the antibody classes. In a preferred embodiment, the Fc variants of the present invention find use in antibodies or Fc fusions that comprise sequences belonging to the IgG class of antibodies, including IgG1, IgG2, IgG3, or IgG4. In an alternate embodiment the Fc variants of the present invention find use in antibodies or Fc fusions that comprise sequences belonging to the IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG, or IgM classes of antibodies. The Fc variants of the present invention may comprise more than one protein chain. That is, the present invention may find use in an antibody or Fc fusion that is a monomer or an oligomer, including a homo- or hetero-oligomer.

[098] The Fc variants of the present invention may be combined with other Fc modifications, including but not limited to modifications that alter effector function. Such combination may provide additive, synergistic, or novel properties in antibodies or Fc fusions. In one embodiment, the Fc variants of the present invention may be combined with other known Fc variants (Duncan *et al.*, 1988, *Nature* 332:563-564; Lund *et al.*, 1991, *J Immunol* 147:2657-2662; Lund *et al.*, 1992, *Mol Immunol* 29:53-59; Alegre *et al.*, 1994, *Transplantation* 57:1537-1543; Hutchins *et al.*, 1995, *Proc Natl Acad Sci U S A* 92:11980-11984; Jefferis *et al.*, 1995, *Immunol Lett* 44:111-117; Lund *et al.*, 1995, *Faseb J* 9:115-119; Jefferis *et al.*, 1996, *Immunol Lett* 54:101-104; Lund *et al.*, 1996, *J Immunol* 157:4963-4969; Armour *et al.*, 1999, *Eur J Immunol* 29:2613-2624; Idusogie *et al.*, 2000, *J Immunol* 164:4178-4184; Reddy *et al.*, 2000, *J Immunol* 164:1925-1933; Xu *et al.*, 2000, *Cell Immunol* 200:16-26; Idusogie *et al.*, 2001, *J Immunol* 166:2571-2575; Shields *et al.*, 2001, *J Biol Chem* 276:6591-6604; Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65; Presta *et al.*, 2002, *Biochem Soc Trans* 30:487-490) (US 5,624,821; US 5,885,573; US 6,194,551; PCT WO 00/42072; PCT WO 99/58572). In an alternate embodiment, the Fc variants of the present invention are incorporated into an antibody or Fc fusion that comprises one or more engineered glycoforms. By "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to an Fc polypeptide, wherein said carbohydrate composition differs chemically from that of a parent Fc polypeptide. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. Engineered glycoforms may be generated by any method, for example by using engineered or variant expression strains, by co-expression with one or more enzymes, for example □1–4- N-acetylglucosaminyltransferase III (GnTIII), by expressing an Fc polypeptide in various organisms or cell lines from various organisms, or by modifying carbohydrate(s) after the Fc polypeptide has been expressed. Methods for generating engineered glycoforms are known in the

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art, and include but are not limited to (Umaña *et al.*, 1999, *Nat Biotechnol* 17:176-180; Davies *et al.*, 2001, *Biotechnol Bioeng* 74:288-294; Shields *et al.*, 2002, *J Biol Chem* 277:26733-26740; Shinkawa *et al.*, 2003, *J Biol Chem* 278:3466-3473) US 6,602,684; USSN 10/277,370; USSN 10/113,929; PCT WO 00/61739A1; PCT WO 01/29246A1; PCT WO 02/31140A1; PCT WO 02/30954A1; Potelligent™ technology (Biowa, Inc., Princeton, N.J.); GlycoMAb™ glycosylation engineering technology (GLYCART biotechnology AG, Zürich, Switzerland)). Engineered glycoform typically refers to the different carbohydrate or oligosaccharide; thus an Fc polypeptide, for example an antibody or Fc fusion, may comprise an engineered glycoform. Alternatively, engineered glycoform may refer to the Fc polypeptide that comprises the different carbohydrate or oligosaccharide. Thus combinations of the Fc variants of the present invention with other Fc modifications, as well as undiscovered Fc modifications, are contemplated with the goal of generating novel antibodies or Fc fusions with optimized properties.

[099] The Fc variants of the present invention may find use in an antibody. By "antibody of the present invention" as used herein is meant an antibody that comprises an Fc variant of the present invention. The present invention may, in fact, find use in any protein that comprises Fc, and thus application of the Fc variants of the present invention is not limited to antibodies. The Fc variants of the present invention may find use in an Fc fusion. By "Fc fusion of the present invention" as used herein refers to an Fc fusion that comprises an Fc variant of the present invention. Fc fusions may comprise an Fc variant of the present invention operably linked to a cytokine, soluble receptor domain, adhesion molecule, ligand, enzyme, peptide, or other protein or protein domain, and include but are not limited to Fc fusions described in US 5,843,725; US 6,018,026; US 6,291,212; US 6,291,646; US 6,300,099; US 6,323,323; PCT WO 00/24782; and in (Chamow *et al.*, 1996, *Trends Biotechnol* 14:52-60; Ashkenazi *et al.*, 1997, *Curr Opin Immunol* 9:195-200).

[100] Virtually any antigen may be targeted by the antibodies and fusions of the present invention, including but are not limited to the following list of proteins, subunits, domains, motifs, and epitopes belonging to the following list of proteins: CD2; CD3, CD3E, CD4, CD11, CD11a, CD14, CD16, CD18, CD19, CD20, CD22, CD23, CD25, CD28, CD29, CD30, CD32, CD33 (p67 protein), CD38, CD40, CD40L, CD52, CD54, CD56, CD80, CD147, GD3, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-6R, IL-8, IL-12, IL-15, IL-18, IL-23, interferon alpha, interferon beta, interferon gamma; TNF-alpha, TNFbeta2, TNFc, TNFalphabeta, TNF-RI, TNF-RII, FasL, CD27L, CD30L, 4-1BBL, TRAIL, RANKL, TWEAK, APRIL, BAFF, LIGHT, VEGI, OX40L, TRAIL Receptor-1, A1 Adenosine Receptor, Lymphotoxin Beta Receptor, TACI, BAFF-R, EPO; LFA-3, ICAM-1, ICAM-3, EpCAM, integrin beta1, integrin beta2, integrin alpha4/beta7, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha5, integrin alpha6, integrin alphav, alphaVbeta3 integrin, FGFR-3, Keratinocyte Growth Factor, VLA-1, VLA-4, L-selectin, anti-Id, E-selectin, HLA, HLA-DR, CTLA-4, T cell receptor, B7-1, B7-2, VNRintegrin, TGFbeta1, TGFbeta2, eotaxin1, BLyS (B-lymphocyte Stimulator), complement C5, IgE, factor VII, CD64, CBL, NCA 90, EGFR (ErbB-1), Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), Tissue

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Factor, VEGF, VEGFR, endothelin receptor, VLA-4, Hapten NP-cap or NIP-cap, T cell receptor alpha/beta, E-selectin, digoxin, placental alkaline phosphatase (PLAP) and testicular PLAP-like alkaline phosphatase, transferrin receptor, Carcinoembryonic antigen (CEA), CEACAM5, HMFG PEM, mucin MUC1, MUC18, Heparanase I, human cardiac myosin, tumor-associated glycoprotein-72 (TAG-72), tumor-associated antigen CA 125, Prostate specific membrane antigen (PSMA), High molecular weight melanoma-associated antigen (HMW-MAA), carcinoma-associated antigen, Gcoprotein IIb/IIIa (GPIIb/IIIa), tumor-associated antigen expressing Lewis Y related carbohydrate, human cytomegalovirus (HCMV) gH envelope glycoprotein, HIV gp120, HCMV, respiratory syncital virus RSV F, RSVF Fgp, VNRintegrin, IL-8, cytokeratin tumor-associated antigen, Hep B gp120, CMV, gpIIbIIIa, HIV IIIB gp120 V3 loop, respiratory syncytial virus (RSV) Fgp, Herpes simplex virus (HSV) gD glycoprotein, HSV gB glycoprotein, HCMV gB envelope glycoprotein, and *Clostridium perfringens* toxin.

[101] One skilled in the art will appreciate that the aforementioned list of targets refers not only to specific proteins and biomolecules, but the biochemical pathway or pathways that comprise them. For example, reference to CTLA-4 as a target antigen implies that the ligands and receptors that make up the T cell co-stimulatory pathway, including CTLA-4, B7-1, B7-2, CD28, and any other undiscovered ligands or receptors that bind these proteins, are also targets. Thus target as used herein refers not only to a specific biomolecule, but the set of proteins that interact with said target and the members of the biochemical pathway to which said target belongs. One skilled in the art will further appreciate that any of the aforementioned target antigens, the ligands or receptors that bind them, or other members of their corresponding biochemical pathway, may be operably linked to the Fc variants of the present invention in order to generate an Fc fusion. Thus for example, an Fc fusion that targets EGFR could be constructed by operably linking an Fc variant to EGF, TGF□, or any other ligand, discovered or undiscovered, that binds EGFR. Accordingly, an Fc variant of the present invention could be operably linked to EGFR in order to generate an Fc fusion that binds EGF, TGF□, or any other ligand, discovered or undiscovered, that binds EGFR. Thus virtually any polypeptide, whether a ligand, receptor, or some other protein or protein domain, including but not limited to the aforementioned targets and the proteins that compose their corresponding biochemical pathways, may be operably linked to the Fc variants of the present invention to develop an Fc fusion.

[102] A number of antibodies and Fc fusions that are approved for use, in clinical trials, or in development may benefit from the Fc variants of the present invention. Said antibodies and Fc fusions are herein referred to as "clinical products and candidates". Thus in a preferred embodiment, the Fc variants of the present invention may find use in a range of clinical products and candidates. For example, a number of antibodies that target CD20 may benefit from the Fc variants of the present invention. For example the Fc variants of the present invention may find use in an antibody that is substantially similar to rituximab (Rituxan®, IDEC/Genentech/Roche) (see for example US 5,736,137), a chimeric anti-CD20 antibody approved to treat Non-Hodgkin's lymphoma; HuMax-

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CD20, an anti-CD20 currently being developed by Genmab, an anti-CD20 antibody described in US 5,500,362, AME-133 (Applied Molecular Evolution), hA20 (Immunomedics, Inc.), and HumaLYM (Intracel). A number of antibodies that target members of the family of epidermal growth factor receptors, including EGFR (ErbB-1), Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), may benefit from the Fc variants of the present invention. For example the Fc variants of the present invention may find use in an antibody that is substantially similar to trastuzumab (Herceptin®, Genentech) (see for example US 5,677,171), a humanized anti-Her2/neu antibody approved to treat breast cancer; pertuzumab (rhuMab-2C4, Omnitarg™), currently being developed by Genentech; an anti-Her2 antibody described in US 4,753,894; cetuximab (Erbitux®, Imclone) (US 4,943,533; PCT WO 96/40210), a chimeric anti-EGFR antibody in clinical trials for a variety of cancers; ABX-EGF (US 6,235,883), currently being developed by Abgenix/Immunex/Amgen; HuMax-EGFr (USSN 10/172,317), currently being developed by Genmab; 425, EMD55900, EMD62000, and EMD72000 (Merck KGaA) (US 5558864; Murthy et al. 1987, *Arch Biochem Biophys.* 252(2):549-60; Rodeck et al., 1987, *J Cell Biochem.* 35(4):315-20; Kettleborough et al., 1991, *Protein Eng.* 4(7):773-83); ICR62 (Institute of Cancer Research) (PCT WO 95/20045; Modjtahedi et al., 1993, *J. Cell Biophys.* 1993, 22(1-3):129-46; Modjtahedi et al., 1993, *Br J Cancer.* 1993, 67(2):247-53; Modjtahedi et al, 1996, *Br J Cancer*, 73(2):228-35; Modjtahedi et al, 2003, *Int J Cancer,* 105(2):273-80); TheraCIM hR3 (YM Biosciences, Canada and Centro de Immunologia Molecular, Cuba (US 5,891,996; US 6, 506,883; Mateo et al, 1997, *Immunotechnology*, 3(1):71-81); mAb-806 (Ludwig Institue for Cancer Research, Memorial Sloan-Kettering) (Jungbluth et al. 2003, *Proc Natl Acad Sci U S A.* 100(2):639-44); KSB-102 (KS Biomedix); MR1-1 (IVAX, National Cancer Institute) (PCT WO 0162931A2); and SC100 (Scancell) (PCT WO 01/88138). In another preferred embodiment, the Fc variants of the present invention may find use in alemtuzumab (Campath®, Millenium), a humanized monoclonal antibody currently approved for treatment of B-cell chronic lymphocytic leukemia. The Fc variants of the present invention may find use in a variety of antibodies or Fc fusions that are substantially similar to other clinical products and candidates, including but not limited to muromonab-CD3 (Orthoclone OKT3®), an anti-CD3 antibody developed by Ortho Biotech/Johnson & Johnson, ibritumomab tiuxetan (Zevalin®), an anti-CD20 antibody developed by IDEC/Schering AG, gemtuzumab ozogamicin (Mylotarg®), an anti-CD33 (p67 protein) antibody developed by Celltech/Wyeth, alefacept (Amevive®), an anti-LFA-3 Fc fusion developed by Biogen), abciximab (ReoPro®), developed by Centocor/Lilly, basiliximab (Simulect®), developed by Novartis, palivizumab (Synagis®), developed by MedImmune, infliximab (Remicade®), an anti-TNFalpha antibody developed by Centocor, adalimumab (Humira®, an anti-TNFalpha antibody developed by Abbott, Humicade™, an anti-TNFalpha antibody developed by Celltech, etanercept (Enbrel®), an anti-TNFalpha Fc fusion developed by Immunex/Amgen, ABX-CBL, an anti-CD147 antibody being developed by Abgenix, ABX-IL8, an anti-IL8 antibody being developed by Abgenix, ABX-MA1, an anti-MUC18 antibody being developed by Abgenix, Pemtumomab (R1549, ^{90}Y-muHMFG1), an anti-MUC1 In development by Antisoma, Therex (R1550), an anti-MUC1 antibody being developed by Antisoma, AngioMab (AS1405), being developed by Antisoma, HuBC-1, being developed by Antisoma, Thioplatin (AS1407) being developed by Antisoma, Antegren® (natalizumab), an anti-alpha-4-beta-1 (VLA-4) and alpha-4-

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beta-7 antibody being developed by Biogen, VLA-1 mAb, an anti-VLA-1 integrin antibody being developed by Biogen, LTBR mAb, an anti-lymphotoxin beta receptor (LTBR) antibody being developed by Biogen, CAT-152, an anti-TGF□2 antibody being developed by Cambridge Antibody Technology, J695, an anti-IL-12 antibody being developed by Cambridge Antibody Technology and Abbott, CAT-192, an anti-TGF□1 antibody being developed by Cambridge Antibody Technology and Genzyme, CAT-213, an anti-Eotaxin1 antibody being developed by Cambridge Antibody Technology, LymphoStat-B™ an anti-Blys antibody being developed by Cambridge Antibody Technology and Human Genome Sciences Inc., TRAIL-R1mAb, an anti-TRAIL-R1 antibody being developed by Cambridge Antibody Technology and Human Genome Sciences, Inc., Avastin™ (bevacizumab, rhuMAb-VEGF), an anti-VEGF antibody being developed by Genentech, an anti-HER receptor family antibody being developed by Genentech, Anti-Tissue Factor (ATF), an anti-Tissue Factor antibody being developed by Genentech, Xolair™ (Omalizumab), an anti-IgE antibody being developed by Genentech, Raptiva™ (Efalizumab), an anti-CD11a antibody being developed by Genentech and Xoma, MLN-02 Antibody (formerly LDP-02), being developed by Genentech and Millenium Pharmaceuticals, HuMax CD4, an anti-CD4 antibody being developed by Genmab, HuMax-IL15, an anti-IL15 antibody being developed by Genmab and Amgen, HuMax-Inflam, being developed by Genmab and Medarex, HuMax-Cancer, an anti-Heparanase I antibody being developed by Genmab and Medarex and Oxford GcoSciences, HuMax-Lymphoma, being developed by Genmab and Amgen, HuMax-TAC, being developed by Genmab, IDEC-131, and anti-CD40L antibody being developed by IDEC Pharmaceuticals, IDEC-151 (Clenoliximab), an anti-CD4 antibody being developed by IDEC Pharmaceuticals, IDEC-114, an anti-CD80 antibody being developed by IDEC Pharmaceuticals, IDEC-152, an anti-CD23 being developed by IDEC Pharmaceuticals, anti-macrophage migration factor (MIF) antibodies being developed by IDEC Pharmaceuticals, BEC2, an anti-idiotypic antibody being developed by Imclone, IMC-1C11, an anti-KDR antibody being developed by Imclone, DC101, an anti-flk-1 antibody being developed by Imclone, anti-VE cadherin antibodies being developed by Imclone, CEA-Cide™ (labetuzumab), an anti-carcinoembryonic antigen (CEA) antibody being developed by Immunomedics, LymphoCide™ (Epratuzumab), an anti-CD22 antibody being developed by Immunomedics, AFP-Cide, being developed by Immunomedics, MyelomaCide, being developed by Immunomedics, LkoCide, being developed by Immunomedics, ProstaCide, being developed by Immunomedics, MDX-010, an anti-CTLA4 antibody being developed by Medarex, MDX-060, an anti-CD30 antibody being developed by Medarex, MDX-070 being developed by Medarex, MDX-018 being developed by Medarex, Osidem™ (IDM-1), and anti-Her2 antibody being developed by Medarex and Immuno-Designed Molecules, HuMax™-CD4, an anti-CD4 antibody being developed by Medarex and Genmab, HuMax-IL15, an anti-IL15 antibody being developed by Medarex and Genmab, CNTO 148, an anti-TNF□ antibody being developed by Medarex and Centocor/J&J, CNTO 1275, an anti-cytokine antibody being developed by Centocor/J&J, MOR101 and MOR102, anti-intercellular adhesion molecule-1 (ICAM-1) (CD54) antibodies being developed by MorphoSys, MOR201, an anti-fibroblast growth factor receptor 3 (FGFR-3) antibody being developed by MorphoSys, Nuvion® (visilizumab), an anti-CD3 antibody being developed by Protein Design Labs, HuZAF™, an anti-gamma interferon antibody being developed by Protein Design Labs, Anti-□5□1

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Integrin, being developed by Protein Design Labs, anti-IL-12, being developed by Protein Design Labs, ING-1, an anti-Ep-CAM antibody being developed by Xoma, and MLN01, an anti-Beta2 integrin antibody being developed by Xoma.

[103] Application of the Fc variants to the aforementioned antibody and Fc fusion clinical products and candidates is not meant to be constrained to their precise composition. The Fc variants of the present invention may be incorporated into the aforementioned clinical candidates and products, or into antibodies and Fc fusions that are substantially similar to them. The Fc variants of the present invention may be incorporated into versions of the aforementioned clinical candidates and products that are humanized, affinity matured, engineered, or modified in some other way. Furthermore, the entire polypeptide of the aforementioned clinical products and candidates need not be used to construct a new antibody or Fc fusion that incorporates the Fc variants of the present invention; for example only the variable region of a clinical product or candidate antibody, a substantially similar variable region, or a humanized, affinity matured, engineered, or modified version of the variable region may be used. In another embodiment, the Fc variants of the present invention may find use in an antibody or Fc fusion that binds to the same epitope, antigen, ligand, or receptor as one of the aforementioned clinical products and candidates.

[104] The Fc variants of the present invention may find use in a wide range of antibody and Fc fusion products. In one embodiment the antibody or Fc fusion of the present invention is a therapeutic, a diagnostic, or a research reagent, preferably a therapeutic. Alternatively, the antibodies and Fc fusions of the present invention may be used for agricultural or industrial uses. In an alternate embodiment, the Fc variants of the present invention compose a library that may be screened experimentally. This library may be a list of nucleic acid or amino acid sequences, or may be a physical composition of nucleic acids or polypeptides that encode the library sequences. The Fc variant may find use in an antibody composition that is monoclonal or polyclonal. In a preferred embodiment, the antibodies and Fc fusions of the present invention are used to kill target cells that bear the target antigen, for example cancer cells. In an alternate embodiment, the antibodies and Fc fusions of the present invention are used to block, antagonize, or agonize the target antigen, for example for antagonizing a cytokine or cytokine receptor. In an alternately preferred embodiment, the antibodies and Fc fusions of the present invention are used to block, antagonize, or agonize the target antigen and kill the target cells that bear the target antigen.

[105] The Fc variants of the present invention may be used for various therapeutic purposes. In a preferred embodiment, the Fc variant proteins are administered to a patient to treat an antibody-related disorder. A "patient" for the purposes of the present invention includes both humans and other animals, preferably mammals and most preferably humans. Thus the antibodies and Fc fusions of the present invention have both human therapy and veterinary applications. In the preferred embodiment

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the patient is a mammal, and in the most preferred embodiment the patient is human. The term "treatment" in the present invention is meant to include therapeutic treatment, as well as prophylactic, or suppressive measures for a disease or disorder. Thus, for example, successful administration of an antibody or Fc fusion prior to onset of the disease results in treatment of the disease. As another example, successful administration of an optimized antibody or Fc fusion after clinical manifestation of the disease to combat the symptoms of the disease comprises treatment of the disease. "Treatment" also encompasses administration of an optimized antibody or Fc fusion protein after the appearance of the disease in order to eradicate the disease. Successful administration of an agent after onset and after clinical symptoms have developed, with possible abatement of clinical symptoms and perhaps amelioration of the disease, comprises treatment of the disease. Those "in need of treatment" include mammals already having the disease or disorder, as well as those prone to having the disease or disorder, including those in which the disease or disorder is to be prevented. By "antibody related disorder" or "antibody responsive disorder" or "condition" or "disease" herein are meant a disorder that may be ameliorated by the administration of a pharmaceutical composition comprising an antibody or Fc fusion of the present invention. Antibody related disorders include but are not limited to autoimmune diseases, immunological diseases, infectious diseases, inflammatory diseases, neurological diseases, and oncological and neoplastic diseases including cancer. By "cancer" and "cancerous" herein 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 (including liposarcoma), neuroendocrine tumors, mesothelioma, schwanoma, meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophagael cancer, tumors of the biliary tract, as well as head and neck cancer. Furthermore, the Fc variants of the present invention may be used to treat conditions including but not limited to congestive heart failure (CHF), vasculitis, rosecea, acne, eczema, myocarditis and other conditions of the myocardium, systemic lupus erythematosus, diabetes, spondylopathies, synovial fibroblasts, and bone marrow stroma; bone loss; Paget's disease, osteoclastoma; multiple myeloma; breast cancer; disuse osteopenia; malnutrition, periodontal disease, Gaucher's disease, Langerhans' cell histiocytosis, spinal cord injury, acute septic arthritis, osteomalacia, Cushing's syndrome, monoostotic fibrous dysplasia, polyostotic fibrous dysplasia, periodontal reconstruction, and bone fractures; sarcoidosis; multiple myeloma; osteolytic bone cancers, breast cancer, lung cancer, kidney cancer and rectal cancer; bone metastasis, bone pain management, and humoral malignant hypercalcemia, ankylosing spondylitisa and other spondyloarthropathies; transplantation rejection, viral infections, hematologic neoplasisas and

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neoplastic-like conditions for example, Hodgkin's lymphoma; non-Hodgkin's lymphomas (Burkitt's lymphoma, small lymphocytic lymphoma/chronic lymphocytic leukemia, mycosis fungoides, mantle cell lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, marginal zone lymphoma, hairy cell leukemia and lymphoplasmacytic leukemia), tumors of lymphocyte precursor cells, including B-cell acute lymphoblastic leukemia/lymphoma, and T-cell acute lymphoblastic leukemia/lymphoma, thymoma, tumors of the mature T and NK cells, including peripheral T-cell leukemias, adult T-cell leukemia/T-cell lymphomas and large granular lymphocytic leukemia, Langerhans cell histocytosis, myeloid neoplasias such as acute myelogenous leukemias, including AML with maturation, AML without differentiation, acute promyelocytic leukemia, acute myelomonocytic leukemia, and acute monocytic leukemias, myelodysplastic syndromes, and chronic myeloproliferative disorders, including chronic myelogenous leukemia, tumors of the central nervous system, e.g., brain tumors (glioma, neuroblastoma, astrocytoma, medulloblastoma, ependymoma, and retinoblastoma), solid tumors (nasopharyngeal cancer, basal cell carcinoma, pancreatic cancer, cancer of the bile duct, Kaposi's sarcoma, testicular cancer, uterine, vaginal or cervical cancers, ovarian cancer, primary liver cancer or endometrial cancer, and tumors of the vascular system (angiosarcoma and hemagiopericytoma), osteoporosis, hepatitis, HIV, AIDS, spondyloarthritis, rheumatoid arthritis, inflammatory bowel diseases (IBD), sepsis and septic shock, Crohn's Disease, psoriasis, schleraderma, graft versus host disease (GVHD), allogenic islet graft rejection, hematologic malignancies, such as multiple myeloma (MM), myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML), inflammation associated with tumors, peripheral nerve injury or demyelinating diseases.

[106] In one embodiment, an antibody or Fc fusion of the present invention is administered to a patient having a disease involving inappropriate expression of a protein. Within the scope of the present invention this is meant to include diseases and disorders characterized by aberrant proteins, due for example to alterations in the amount of a protein present, the presence of a mutant protein, or both. An overabundance may be due to any cause, including but not limited to overexpression at the molecular level, prolonged or accumulated appearance at the site of action, or increased activity of a protein relative to normal. Included within this definition are diseases and disorders characterized by a reduction of a protein. This reduction may be due to any cause, including but not limited to reduced expression at the molecular level, shortened or reduced appearance at the site of action, mutant forms of a protein, or decreased activity of a protein relative to normal. Such an overabundance or reduction of a protein can be measured relative to normal expression, appearance, or activity of a protein, and said measurement may play an important role in the development and/or clinical testing of the antibodies and Fc fusions of the present invention.

[107] In one embodiment, an antibody or Fc fusion of the present invention is the only therapeutically active agent administered to a patient. Alternatively, the antibody or Fc fusion of the present invention is administered in combination with one or more other therapeutic agents, including but not limited to cytotoxic agents, chemotherapeutic agents, cytokines, growth inhibitory agents, anti-

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hormonal agents, kinase inhibitors, anti-angiogenic agents, cardioprotectants, or other therapeutic agents. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The skilled medical practitioner can determine empirically the appropriate dose or doses of other therapeutic agents useful herein. The antibodies and Fc fusions of the present invention may be administered concomitantly with one or more other therapeutic regimens. For example, an antibody or Fc fusion of the present invention may be administered to the patient along with chemotherapy, radiation therapy, or both chemotherapy and radiation therapy. In one embodiment, the antibody or Fc fusion of the present invention may be administered in conjunction with one or more antibodies or Fc fusions, which may or may not comprise an Fc variant of the present invention.

[108] In one embodiment, the antibodies and Fc fusions of the present invention are administered with a chemotherapeutic agent. By "chemotherapeutic agent" as used herein is meant a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include but are not limited to 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, caminomycin, 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; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofuran; 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

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Oncology, Princeton, N.J.) and docetaxel (TAXOTERE®, Rhne-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; thymidylate synthase inhibitor (such as Tomudex); cox-2 inhibitors, such as celicoxib (CELEBREX®) or MK-0966 (VIOXX®); 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.

[109] A chemotherapeutic or other cytotoxic agent may be administered as a prodrug. By "prodrug" as used herein is meant 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, for example Wilman, 1986, Biochemical Society Transactions, 615th Meeting Belfast, 14:375-382; and Stella *et al.*, "Prodrugs: A Chemical Approach to Targeted Drug Delivery," Directed Drug Delivery, Borchardt *et al.*, (ed.): 247-267, Humana Press, 1985. The prodrugs that may find use with the present 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, beta-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 with the antibodies and Fc fusions of the present invention include but are not limited to any of the aforementioned chemotherapeutic agents.

[110] The antibodies and Fc fusions of the present invention may be combined with other therapeutic regimens. For example, in one embodiment, the patient to be treated with the antibody or Fc fusion may also receive radiation therapy. Radiation therapy can be administered according to protocols commonly employed in the art and known to the skilled artisan. Such therapy includes but is not limited to cesium, iridium, iodine, or cobalt radiation. The radiation therapy may be whole body irradiation, or may be directed locally to a specific site or tissue in or on the body, such as the lung, bladder, or prostate. Typically, radiation therapy is administered in pulses over a period of time from about 1 to 2 weeks. The radiation therapy may, however, be administered over longer periods of time. For instance, radiation therapy may be administered to patients having head and neck cancer for about 6 to about 7 weeks. Optionally, the radiation therapy may be administered as a single dose

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or as multiple, sequential doses. The skilled medical practitioner can determine empirically the appropriate dose or doses of radiation therapy useful herein. In accordance with another embodiment of the invention, the antibody or Fc fusion of the present invention and one or more other anti-cancer therapies are employed to treat cancer cells *ex vivo*. It is contemplated that such *ex vivo* treatment may be useful in bone marrow transplantation and particularly, autologous bone marrow transplantation. For instance, treatment of cells or tissue(s) containing cancer cells with antibody or Fc fusion and one or more other anti-cancer therapies, such as described above, can be employed to deplete or substantially deplete the cancer cells prior to transplantation in a recipient patient. It is of course contemplated that the antibodies and Fc fusions of the invention can be employed in combination with still other therapeutic techniques such as surgery.

[111] In an alternate embodiment, the antibodies and Fc fusions of the present invention are administered with a cytokine. By "cytokine" as used herein is meant a generic term for proteins released by one cell population that 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-alpha and -beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; 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-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; 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.

[112] A variety of other therapeutic agents may find use for administration with the antibodies and Fc fusions of the present invention. In one embodiment, the antibody or Fc fusion is administered with an anti-angiogenic agent. By "anti-angiogenic agent" as used herein is meant a compound that blocks, or interferes to some degree, the development of blood vessels. The anti-angiogenic factor may, for instance, be a small molecule or a protein, for example an antibody, Fc fusion, or cytokine, that binds to a growth factor or growth factor receptor involved in promoting angiogenesis. The preferred anti-angiogenic factor herein is an antibody that binds to Vascular Endothelial Growth Factor (VEGF). In an alternate embodiment, the antibody or Fc fusion is administered with a therapeutic

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agent that induces or enhances adaptive immune response, for example an antibody that targets CTLA-4. In an alternate embodiment, the antibody or Fc fusion is administered with a tyrosine kinase inhibitor. By "tyrosine kinase inhibitor" as used herein is meant a molecule that inhibits to some extent tyrosine kinase activity of a tyrosine kinase. Examples of such inhibitors include but are not limited to quinazolines, such as PD 153035, 4-(3-chloroanilino) quinazoline; pyridopyrimidines; pyrimidopyrimidines; pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo(2,3-d) pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lambert); antisense molecules (e.g. those that bind to ErbB-encoding nucleic acid); quinoxalines (US 5,804,396); tryphostins (US 5,804,396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering A G); pan-ErbB inhibitors such as C1-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); Imatinib mesylate (STI571,Gleevec®; Novartis); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); C1-1033 (Pfizer); EKB-569 (Wyeth); Semaxinib (Sugen); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone); or as described in any of the following patent publications: US 5,804,396; PCT WO 99/09016 (American Cyanimid); PCT WO 98/43960 (American Cyanamid); PCT WO 97/38983 (Warner-Lambert); PCT WO 99/06378 (Warner-Lambert); PCT WO 99/06396 (Warner-Lambert); PCT WO 96/30347 (Pfizer, Inc); PCT WO 96/33978 (AstraZeneca); PCT WO96/3397 (AstraZeneca); PCT WO 96/33980 (AstraZeneca), gefitinib (IRESSA™, ZD1839, AstraZeneca), and OSI-774 (Tarceva™, OSI Pharmaceuticals/Genentech).

[113] In an alternate embodiment, the antibody or Fc fusion of the present invention is conjugated or operably linked to another therapeutic compound. The therapeutic compound may be a cytotoxic agent, a chemotherapeutic agent, a toxin, a radioisotope, a cytokine, or other therapeutically active agent. Conjugates of the antibody or Fc fusion 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 tolyene 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., 1971, Science 238:1098. Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See PCT WO 94/11026. 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., 1992, Cancer Research 52: 127-131) may be used. Alternatively, the antibody or Fc fusion is operably linked to the therapeutic agent, e.g. by recombinant techniques or peptide synthesis.

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[114] Chemotherapeutic agents that may be useful for conjugation to the antibodies and Fc fusions of the present invention have been described above. In an alternate embodiment, the antibody or Fc fusion is conjugated or operably linked to a toxin, including but not limited to small molecule toxins and enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Small molecule toxins include but are not limited to calicheamicin, maytansine (US 5,208,020), trichothene, and CC1065. In one embodiment of the invention, the antibody or Fc fusion is conjugated to one or more maytansine molecules (e.g. about 1 to about 10 maytansine molecules per antibody molecule). Maytansine may, for example, be converted to May-SS-Me which may be reduced to May-SH3 and reacted with modified antibody or Fc fusion (Chari *et al.*, 1992, *Cancer Research* 52: 127-131) to generate a maytansinoid-antibody or maytansinoid-Fc fusion conjugate. Another conjugate of interest comprises an antibody or Fc fusion 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 that may be used include but are not limited to γ_1^1, $\square_2^1$, $\square_3$, N-acetyl-γ_1^1, PSAG, and Θ^1_1, (Hinman *et al.*, 1993, *Cancer Research* 53:3336-3342; Lode *et al.*, 1998, *Cancer Research* 58:2925-2928) (US 5,714,586; US 5,712,374; US 5,264,586; US 5,773,001). Dolastatin 10 analogs such as auristatin E (AE) and monomethylauristatin E (MMAE) may find use as conjugates for the Fc variants of the present invention (Doronina *et al.*, 2003, *Nat Biotechnol* 21(7):778-84; Francisco *et al.*, 2003 *Blood* 102(4):1458-65). Useful enyzmatically active toxins include but are not limited to 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, PCT WO 93/21232. The present invention further contemplates a conjugate or fusion formed between an antibody or Fc fusion of the present invention and a compound with nucleolytic activity, for example a ribonuclease or DNA endonuclease such as a deoxyribonuclease (DNase).

[115] In an alternate embodiment, an antibody or Fc fusion of the present invention may be conjugated or operably linked to a radioisotope to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugate antibodies and Fc fusions. Examples include, but are not limited to, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, and radioactive isotopes of Lu.

[116] In yet another embodiment, an antibody or Fc fusion of the present invention may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor or Fc fusion-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide). In an alternate

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embodiment, the antibody or Fc fusion is conjugated or operably linked to an enzyme in order to employ Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT). ADEPT may be used by conjugating or operably linking the antibody or Fc fusion to a prodrug-activating enzyme that converts a prodrug (e.g. a peptidyl chemotherapeutic agent, see PCT WO 81/01145) to an active anti-cancer drug. See, for example, PCT WO 88/07378 and US 4,975,278. The enzyme component of the immunoconjugate useful for ADEPT 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 .beta.-galactosidase and neuramimidase useful for converting glycosylated prodrugs into free drugs; beta-lactamase useful for converting drugs derivatized.with .alpha.-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, for example, Massey, 1987, *Nature* 328: 457-458). Antibody-abzyme and Fc fusion-abzyme conjugates can be prepared for delivery of the abzyme to a tumor cell population. Other modifications of the antibodies and Fc fusions of the present invention are contemplated herein. For example, the antibody or Fc fusion 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.

[117] Pharmaceutical compositions are contemplated wherein an antibody or Fc fusion of the present invention and and one or more therapeutically active agents are formulated. Formulations of the antibodies and Fc fusions of the present invention are prepared for storage by mixing said antibody or Fc fusion 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, acetate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl orbenzyl 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;

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monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; sweeteners and other flavoring agents; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; additives; coloring agents; 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). In a preferred embodiment, the pharmaceutical composition that comprises the antibody or Fc fusion of the present invention is in a water-soluble form, such as being present as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. "Pharmaceutically acceptable acid addition salt" refers to those salts that retain the biological effectiveness of the free bases and that are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. "Pharmaceutically acceptable base addition salts" include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. The formulations to be used for *in vivo* administration are preferrably sterile. This is readily accomplished by filtration through sterile filtration membranes or other methods.

[118] The antibodies and Fc fusions disclosed herein may also be formulated as immunoliposomes. A liposome is a small vesicle comprising various types of lipids, phospholipids and/or surfactant that is useful for delivery of a therapeutic agent to a mammal. Liposomes containing the antibody or Fc fusion are prepared by methods known in the art, such as described in Epstein *et al.*, 1985, *Proc Natl Acad Sci USA*, 82:3688; Hwang *et al.*, 1980, *Proc Natl Acad Sci USA*, 77:4030; US 4,485,045; US 4,544,545; and PCT WO 97/38731. Liposomes with enhanced circulation time are disclosed in US 5,013,556. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. 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. A chemotherapeutic agent or other therapeutically active agent is optionally contained within the liposome (Gabizon *et al.*, 1989, *J National Cancer Inst* 81:1484).

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[119] The antibodies, Fc fusions, and other therapeutically active agents may also be entrapped in microcapsules prepared by methods including but not limited to coacervation techniques, interfacial polymerization (for example using hydroxymethylcellulose or gelatin-microcapsules, or poly-(methylmethacylate) microcapsules), colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), and 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 polymer, 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 (US 3,773,919), copolymers of L-glutamic acid and gamma ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (which are injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), poly-D-(-)-3-hydroxybutyric acid, and ProLease® (commercially available from Alkermes), which is a microsphere-based delivery system composed of the desired bioactive molecule incorporated into a matrix of poly-DL-lactide-co-glycolide (PLG).

[120] The concentration of the therapeutically active antibody or Fc fusion of the present invention in the formulation may vary from about 0.1 to 100 weight %. In a preferred embodiment, the concentration of the antibody or Fc fusion is in the range of 0.003 to 1.0 molar. In order to treat a patient, a therapeutically effective dose of the antibody or Fc fusion of the present invention may be administered. By "therapeutically effective dose" herein is meant a dose that produces the effects 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. Dosages may range from 0.01 to 100 mg/kg of body weight or greater, for example 0.1, 1, 10, or 50 mg/kg of body weight, with 1 to 10mg/kg being preferred. As is known in the art, adjustments for antibody or Fc fusion degradation, systemic versus localized delivery, and rate of new protease synthesis, as well as the age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

[121] Administration of the pharmaceutical composition comprising an antibody or Fc fusion of the present invention, preferably in the form of a sterile aqueous solution, may be done in a variety of ways, including, but not limited to, orally, subcutaneously, intravenously, intranasally, intraotically, transdermally, topically (e.g., gels, salves, lotions, creams, etc.), intraperitoneally, intramuscularly, intrapulmonary (e.g., AERx® inhalable technology commercially available from Aradigm, or Inhance™ pulmonary delivery system commercially available from Inhale Therapeutics), vaginally, parenterally, rectally, or intraocularly. In some instances, for example for the treatment of wounds, inflammation, etc., the antibody or Fc fusion may be directly applied as a solution or spray. As is known in the art,

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the pharmaceutical composition may be formulated accordingly depending upon the manner of introduction.

Engineering Methods

[122] The present invention provides engineering methods that may be used to generate Fc variants. A principal obstacle that has hindered previous attempts at Fc engineering is that only random attempts at modification have been possible, due in part to the inefficiency of engineering strategies and methods, and to the low-throughput nature of antibody production and screening. The present invention describes engineering methods that overcome these shortcomings. A variety of design strategies, computational screening methods, library generation methods, and experimental production and screening methods are contemplated. These strategies, approaches, techniques, and methods may be applied individually or in various combinations to engineer optimized Fc variants.

Design Strategies

[123] The most efficient approach to generating Fc variants that are optimized for a desired property is to direct the engineering efforts toward that goal. Accordingly, the present invention teaches design strategies that may be used to engineer optimized Fc variants. The use of a design strategy is meant to guide Fc engineering, but is not meant to constrain an Fc variant to a particular optimized property based on the design strategy that was used to engineer it. At first thought this may seem counterintuitive; however its validity is derived from the enormous complexity of subtle interactions that determine the structure, stability, solubility, and function of proteins and protein-protein complexes. Although efforts can be made to predict which protein positions, residues, interactions, etc. are important for a design goal, often times critical ones are not predictable. Effects on protein structure, stability, solubility, and function, whether favorable or unfavorable, are often unforeseen. Yet there are innumerable amino acid modifications that are detrimental or deleterious to proteins. Thus often times the best approach to engineering comes from generation of protein variants that are focused generally towards a design goal but do not cause detrimental effects. In this way, a principal objective of a design strategy may be the generation of quality diversity. At a simplistic level this can be thought of as stacking the odds in one's favor. As an example, perturbation of the Fc carbohydrate or a particular domain-domain angle, as described below, are valid design strategies for generating optimized Fc variants, despite the fact that how carbohydrate and domain-domain angles determine the properties of Fc is not well understood. By reducing the number of detrimental amino acid modifications that are screened, i.e. by screening quality diversity, these design strategies become practical. Thus the true value of the design strategies taught in the present invention is their ability to direct engineering efforts towards the generation of valuable Fc variants. The specific value of any one resulting variant is determined after experimentation.

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[124] One design strategy for engineering Fc variants is provided in which interaction of Fc with some Fc ligand is altered by engineering amino acid modifications at the interface between Fc and said Fc ligand. Fc ligands herein may include but are not limited to FcγRs, C1q, FcRn, protein A or G, and the like. By exploring energetically favorable substitutions at Fc positions that impact the binding interface, variants can be engineered that sample new interface conformations, some of which may improve binding to the Fc ligand, some of which may reduce Fc ligand binding, and some of which may have other favorable properties. Such new interface conformations could be the result of, for example, direct interaction with Fc ligand residues that form the interface, or indirect effects caused by the amino acid modifications such as perturbation of side chain or backbone conformations. Variable positions may be chosen as any positions that are believed to play an important role in determining the conformation of the interface. For example, variable positions may be chosen as the set of residues that are within a certain distance, for example 5 Angstroms (Å), preferrably between 1 and 10 Å, of any residue that makes direct contact with the Fc ligand.

[125] An additional design strategy for generating Fc variants is provided in which the conformation of the Fc carbohydrate at N297 is optimized. Optimization as used in this context is meant to includes conformational and compositional changes in the N297 carbohydrate that result in a desired property, for example increased or reduced affinity for an FcγR. Such a strategy is supported by the observation that the carbohydrate structure and conformation dramatically affect Fc/FcγR and Fc/C1q binding (Umaña *et al.*, 1999, *Nat Biotechnol* 17:176-180; Davies *et al.*, 2001, *Biotechnol Bioeng* 74:288-294; Mimura *et al.*, 2001, *J Biol Chem* 276:45539-45547.; Radaev *et al.*, 2001, *J Biol Chem* 276:16478-16483; Shields *et al.*, 2002, *J Biol Chem* 277:26733-26740; Shinkawa *et al.*, 2003, *J Biol Chem* 278:3466-3473). However the carbohydrate makes no specific contacts with FcγRs. By exploring energetically favorable substitutions at positions that interact with carbohydrate, a quality diversity of variants can be engineered that sample new carbohydrate conformations, some of which may improve and some of which may reduce binding to one or more Fc ligands. While the majority of mutations near the Fc/carbohydrate interface appear to alter carbohydrate conformation, some mutations have been shown to alter the glycosylation composition (Lund *et al.*, 1996, *J Immunol* 157:4963-4969; Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65).

[126] Another design strategy for generating Fc variants is provided in which the angle between the Cγ2 and Cγ3 domains is optimized Optimization as used in this context is meant to describe conformational changes in the Cγ2-Cγ3 domain angle that result in a desired property, for example increased or reduced affinity for an FcγR. This angle is an important determinant of Fc/FcγR affinity (Radaev *et al.*, 2001, *J Biol Chem* 276:16478-16483), and a number of mutations distal to the Fc/FcγR interface affect binding potentially by modulating it (Shields *et al.*, 2001, *J Biol Chem* 276:6591-6604). By exploring energetically favorable substitutions positions that appear to play a key role in determining the Cγ2-Cγ3 angle and the flexibility of the domains relative to one another, a

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quality diversity of variants can be designed that sample new angles and levels of flexibility, some of which may be optimized for a desired Fc property.

[127] Another design strategy for generating Fc variants is provided in which Fc is reengineered to eliminate the structural and functional dependence on glycosylation. This design strategy involves the optimization of Fc structure, stability, solubility, and/or Fc function (for example affinity of Fc for one or more Fc ligands) in the absence of the N297 carbohydrate. In one approach, positions that are exposed to solvent in the absence of glycosylation are engineered such that they are stable, structurally consistent with Fc structure, and have no tendency to aggregate. The Cγ2 is the only unpaired Ig domain in the antibody (see Figure 1). Thus the N297 carbohydrate covers up the exposed hydrophobic patch that would normally be the interface for a protein-protein interaction with another Ig domain, maintaining the stability and structural integrity of Fc and keeping the Cγ2 domains from aggregating across the central axis. Approaches for optimizing aglycosylated Fc may involve but are not limited to designing amino acid modifications that enhance aglycoslated Fc stability and/or solubility by incorporating polar and/or charged residues that face inward towards the Cγ2-Cγ2 dimer axis, and by designing amino acid modifications that directly enhance the aglycosylated Fc/FcγR interface or the interface of aglycosylated Fc with some other Fc ligand.

[128] An additional design strategy for engineering Fc variants is provided in which the conformation of the Cγ2 domain is optimized Optimization as used in this context is meant to describe conformational changes in the Cγ2 domain angle that result in a desired property, for example increased or reduced affinity for an FcγR. By exploring energetically favorable substitutions at Cγ2 positions that impact the Cγ2 conformation, a quality diversity of variants can be engineered that sample new Cγ2 conformations, some of which may achieve the design goal. Such new Cγ2 conformations could be the result of, for example, alternate backbone conformations that are sampled by the variant. Variable positions may be chosen as any positions that are believed to play an important role in determining C□2 structure, stability, solubility, flexibility, function, and the like. For example, Cγ2 hydrophobic core residues, that is Cγ2 residues that are partially or fully sequestered from solvent, may be reengineered. Alternatively, noncore residues may be considered, or residues that are deemed important for determining backbone structure, stability, or flexibility.

[129] An additional design strategy for Fc optimization is provided in which binding to an FcγR, complement, or some other Fc ligand is altered by modifications that modulate the electrostatic interaction between Fc and said Fc ligand. Such modifications may be thought of as optimization of the global electrostatic character of Fc, and include replacement of neutral amino acids with a charged amino acid, replacement of a charged amino acid with a neutral amino acid, or replacement of a charged amino acid with an amino acid of opposite charge (i.e. charge reversal). Such

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modifications may be used to effect changes in binding affinity between an Fc and one or more Fc ligands, for example FcγRs. In a preferred embodiment, positions at which electrostatic substitutions might affect binding are selected using one of a variety of well known methods for calculation of electrostatic potentials. In the simplest embodiment, Coulomb's law is used to generate electrostatic potentials as a function of the position in the protein. Additional embodiments include the use of Debye-Huckel scaling to account for ionic strength effects, and more sophisticated embodiments such as Poisson-Boltzmann calculations. Such electrostatic calculations may highlight positions and suggest specific amino acid modifications to achieve the design goal. In some cases, these substitutions may be anticipated to variably affect binding to different Fc ligands, for example to enhance binding to activating FcγRs while decreasing binding affinity to inhibitory FcγRs.

Computational Screening

[130] A principal obstacle to obtaining valuable Fc variants is the difficulty in predicting what amino acid modifications, out of the enormous number of possibilities, will achieve the desired goals. Indeed one of the principle reasons that previous attempts at Fc engineering have failed to produce Fc variants of significant clinical value is that approaches to Fc engineering have thus far involved hit-or-miss approaches. The present invention provides computational screening methods that enable quantitative and systematic engineering of Fc variants. These methods typically use atomic level scoring functions, side chain rotamer sampling, and advanced optimization methods to accurately capture the relationships between protein sequence, structure, and function. Computational screening enables exploration of the entire sequence space of possibilities at target positions by filtering the enormous diversity which results. Variant libraries that are screened computationally are effectively enriched for stable, properly folded, and functional sequences, allowing active optimization of Fc for a desired goal. Because of the overlapping sequence constraints on protein structure, stability, solubility, and function, a large number of the candidates in a library occupy "wasted" sequence space. For example, a large fraction of sequence space encodes unfolded, misfolded, incompletely folded, partially folded, or aggregated proteins. This is particularly relevant for Fc engineering because Ig domains are small beta sheet structures, the engineering of which has proven extremely demanding (Quinn *et al.*, 1994, *Proc Natl Acad Sci U S A* 91:8747-8751; Richardson *et al.*, 2002, *Proc Natl Acad Sci U S A* 99:2754-2759). Even seemingly harmless substitutions on the surface of a beta sheet can cause severe packing conflicts, dramatically disrupting folding equilibrium (Smith *et al.*, 1995, *Science* 270:980-982); incidentally, alanine is one of the worst beta sheet formers (Minor *et al.*, 1994, *Nature* 371:264-267). The determinants of beta sheet stability and specificity are a delicate balance between an extremely large number of subtle interactions. Computational screening enables the generation of libraries that are composed primarily of productive sequence space, and as a result increases the chances of identifying proteins that are optimized for the design goal. In effect, computational screening yields an increased hit-rate, thereby decreasing the number of variants that must be screened experimentally. An additional obstacle to Fc engineering is the need for active design of correlated or coupled mutations. For example, the greatest Fc/FcγR affinity

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enhancement observed thus far is S298A/E333A/K334A, obtained by combining three better binders obtained separately in an alanine scan (Shields *et al.*, 2001, *J Biol Chem* 276:6591-6604). Computational screening is capable of generating such a three-fold variant in one experiment instead of three separate ones, and furthermore is able to test the functionality of all 20 amino acids at those positions instead of just alanine. Computational screening deals with such complexity by reducing the combinatorial problem to an experimentally tractable size.

[131] Computational screening, viewed broadly, has four steps: 1) selection and preparation of the protein template structure or structures, 2) selection of variable positions, amino acids to be considered at those positions, and/or selection of rotamers to model considered amino acids, 3) energy calculation, and 4) combinatorial optimization. In more detail, the process of computational screening can be described as follows. A three-dimensional structure of a protein is used as the starting point. The positions to be optimized are identified, which may be the entire protein sequence or subset(s) thereof. Amino acids that will be considered at each position are selected. In a preferred embodiment, each considered amino acid may be represented by a discrete set of allowed conformations, called rotamers. Interaction energies are calculated between each considered amino acid and each other considered amino acid, and the rest of the protein, including the protein backbone and invariable residues. In a preferred embodiment, interaction energies are calculated between each considered amino acid side chain rotamer and each other considered amino acid side chain rotamer and the rest of the protein, including the protein backbone and invariable residues. One or more combinatorial search algorithms are then used to identify the lowest energy sequence and/or low energy sequences.

[132] In a preferred embodiment, the computational screening method used is substantially similar to Protein Design Automation® (PDA®) technology, as is described in US 6,188,965; US 6,269,312; US 6,403,312; USSN 09/782,004; USSN 09/927,790; USSN 10/218,102; PCT WO 98/07254; PCT WO 01/40091; and PCT WO 02/25588. In another preferred embodiment, a computational screening method substantially similar to Sequence Prediction Algorithm™ (SPA™) technology is used, as is described in (Raha *et al.*, 2000, *Protein Sci* 9:1106-1119), USSN 09/877,695, and USSN 10/071,859. In another preferred embodiment, the computational screening methods described in USSN 10/339788, filed on March 3, 2003, entitled "ANTIBODY OPTIMIZATION", are used. In some embodiments, combinations of different computational screening methods are used, including combinations of PDA® technology and SPA™ technology, as well as combinations of these computational methods in combination with other design tools. Similarly, these computational methods can be used simultaneously or sequentially, in any order.

[133] A template structure is used as input into the computational screening calculations. By "template structure" herein is meant the structural coordinates of part or all of a protein to be

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optimized. The template structure may be any protein for which a three dimensional structure (that is, three dimensional coordinates for a set of the protein's atoms) is known or may be calculated, estimated, modeled, generated, or determined. The three dimensional structures of proteins may be determined using methods including but not limited to X-ray crystallographic techniques, nuclear magnetic resonance (NMR) techniques, *de novo* modeling, and homology modeling. If optimization is desired for a protein for which the structure has not been solved experimentally, a suitable structural model may be generated that may serve as the template for computational screening calculations. Methods for generating homology models of proteins are known in the art, and these methods find use in the present invention. See for example, Luo, *et al.* 2002, *Protein Sci* 11: 1218-1226, Lehmann & Wyss, 2001, *Curr Opin Biotechnol* 12(4):371-5.; Lehmann *et al.*, 2000, Biochim Biophys Acta 1543(2):408-415; Rath & Davidson, 2000, *Protein Sci*, 9(12):2457-69; Lehmann *et al.*, 2000, *Protein Eng* 13(1):49-57; Desjarlais & Berg, 1993, *Proc Natl Acad Sci USA* 90(6):2256-60; Desjarlais & Berg, 1992, *Proteins* 12(2):101-4; Henikoff & Henikoff, 2000, *Adv Protein Chem* 54:73-97; Henikoff & Henikoff, 1994, *J Mol Biol* 243(4):574-8; Morea *et al.*, 2000, *Methods* 20:267-269. Protein/protein complexes may also be obtained using docking methods. Suitable protein structures that may serve as template structures include, but are not limited to, all of those found in the Protein Data Base compiled and serviced by the Research Collaboratory for Structural Bioinformatics (RCSB, formerly the Brookhaven National Lab).

[134] The template structure may be of a protein that occurs naturally or is engineered. The template structure may be of a protein that is substantially encoded by a protein from any organism, with human, mouse, rat, rabbit, and monkey preferred. The template structure may comprise any of a number of protein structural forms. In a preferred embodiment the template structure comprises an Fc region or a domain or fragment of Fc. In an alternately preferred embodiment the template structure comprises Fc or a domain or fragment of Fc bound to one or more Fc ligands, with an Fc/FcγR complex being preferred. The Fc in the template structure may be glycosylated or unglycosylated. The template structure may comprise more than one protein chain. The template structure may additionally contain nonprotein components, including but not limited to small molecules, substrates, cofactors, metals, water molecules, prosthetic groups, polymers and carbohydrates. In a preferred embodiment, the template structure is a plurality or set of template proteins, for example an ensemble of structures such as those obtained from NMR. Alternatively, the set of template structures is generated from a set of related proteins or structures, or artificially created ensembles. The composition and source of the template structure depends on the engineering goal. For example, for enhancement of human Fc/FcγR affinity, a human Fc/FcγR complex structure or derivative thereof may be used as the template structure. Alternatively, the uncomplexed Fc structure may be used as the template structure. If the goal is to enhance affinity of a human Fc for a mouse FcγR, the template structure may be a structure or model of a human Fc bound to a mouse FcγR.

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[135] The template structure may be modified or altered prior to design calculations. A variety of methods for template structure preparation are described in US 6,188,965; US 6,269,312; US 6,403,312; USSN 09/782,004; USSN 09/927,790; USSN 09/877,695; USSN 10/071,859, USSN 10/218,102; PCT WO 98/07254; PCT WO 01/40091; and PCT WO 02/25588. For example, in a preferred embodiment, explicit hydrogens may be added if not included within the structure. In an alternate embodiment, energy minimization of the structure is run to relax strain, including strain due to van der Waals clashes, unfavorable bond angles, and unfavorable bond lengths. Alternatively, the template structure is altered using other methods, such as manually, including directed or random perturbations. It is also possible to modify the template structure during later steps of computational screening, including during the energy calculation and combinatorial optimization steps. In an alternate embodiment, the template structure is not modified before or during computational screening calculations.

[136] Once a template structure has been obtained, variable positions are chosen. By "variable position" herein is meant a position at which the amino acid identity is allowed to be altered in a computational screening calculation. As is known in the art, allowing amino acid modifications to be considered only at certain variable positions reduces the complexity of a calculation and enables computational screening to be more directly tailored for the design goal. One or more residues may be variable positions in computational screening calculations. Positions that are chosen as variable positions may be those that contribute to or are hypothesized to contribute to the protein property to be optimized, for example Fc affinity for an FcγR, Fc stability, Fc solubility, and so forth. Residues at variable positions may contribute favorably or unfavorably to a specific protein property. For example, a residue at an Fc/FcγR interface may be involved in mediating binding, and thus this position may be varied in design calculations aimed at improving Fc/FcγR affinity. As another example, a residue that has an exposed hydrophobic side chain may be responsible for causing unfavorable aggregation, and thus this position may be varied in design calculations aimed at improving solubility. Variable positions may be those positions that are directly involved in interactions that are determinants of a particular protein property. For example, the Fc□R binding site of Fc may be defined to include all residues that contact that particular FγcR. By "contact" herein is meant some chemical interaction between at least one atom of an Fc residue with at least one atom of the bound FcγR, with chemical interaction including, but not limited to van der Waals interactions, hydrogen bond interactions, electrostatic interactions, and hydrophobic interactions. In an alternative embodiment, variable positions may include those positions that are indirectly involved in a protein property, i.e. such positions may be proximal to residues that are known to or hypothesized to contribute to an Fc property. For example, the FcγR binding site of an Fc may be defined to include all Fc residues within a certain distance, for example 4 - 10 Å, of any Fc residue that is in van der Waals contact with the FcγR. Thus variable positions in this case may be chosen not only as residues that directly contact the FcγR, but also those that contact residues that contact the FcγR and thus influence binding indirectly. The specific positions chosen are dependent on the design strategy being employed.

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[137] One or more positions in the template structure that are not variable may be floated. By "floated position" herein is meant a position at which the amino acid conformation but not the amino acid identity is allowed to vary in a computational screening calculation. In one embodiment, the floated position may have the parent amino acid identity. For example, floated positions may be positions that are within a small distance, for example 5 Å, of a variable position residue. In an alternate embodiment, a floated position may have a non-parent amino acid identity. Such an embodiment may find use in the present invention, for example, when the goal is to evaluate the energetic or structural outcome of a specific mutation.

[138] Positions that are not variable or floated are fixed. By "fixed position" herein is meant a position at which the amino acid identity and the conformation are held constant in a computational screening calculation. Positions that may be fixed include residues that are not known to be or hypothesized to be involved in the property to be optimized. In this case the assumption is that there is little or nothing to be gained by varying these positions. Positions that are fixed may also include positions whose residues are known or hypothesized to be important for maintaining proper folding, structure, stability, solubility, and/or biological function. For example, positions may be fixed for residues that interact with a particular Fc ligand or residues that encode a glycosylation site in order to ensure that binding to the Fc ligand and proper glycosylation respectively are not perturbed. Likewise, if stability is being optimized, it may be beneficial to fix positions that directly or indirectly interact with an Fc ligand, for example an Fc□R, so that binding is not perturbed. Fixed positions may also include structurally important residues such as cysteines participating in disulfide bridges, residues critical for determining backbone conformation such as proline or glycine, critical hydrogen bonding residues, and residues that form favorable packing interactions.

[139] The next step in computational screening is to select a set of possible amino acid identities that will be considered at each particular variable position. This set of possible amino acids is herein referred to as "considered amino acids" at a variable position. "Amino acids" as used herein refers to the set of natural 20 amino acids and any nonnatural or synthetic analogues. In one embodiment, all 20 natural amino acids are considered. Alternatively, a subset of amino acids, or even only one amino acid is considered at a given variable position. As will be appreciated by those skilled in the art, there is a computational benefit to considering only certain amino acid identities at variable positions, as it decreases the combinatorial complexity of the search. Furthermore, considering only certain amino acids at variable positions may be used to tailor calculations toward specific design strategies. For example, for solubility optimization of aglycosylated Fc, it may be beneficial to allow only polar amino acids to be considered at nonpolar Fc residues that are exposed to solvent in the absence of carbohydrate. Nonnatural amino acids, including synthetic amino acids and analogues of natural amino acids, may also be considered amino acids. For example see Chin *et al.*, 2003, *Science*, 301(5635):964-7; and Chin *et al.*, 2003, *Chem Biol.*10(6):511-9.

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[140] A wide variety of methods may be used, alone or in combination, to select which amino acids will be considered at each position. For example, the set of considered amino acids at a given variable position may be chosen based on the degree of exposure to solvent. Hydrophobic or nonpolar amino acids typically reside in the interior or core of a protein, which are inaccessible or nearly inaccessible to solvent. Thus at variable core positions it may be beneficial to consider only or mostly nonpolar amino acids such as alanine, valine, isoleucine, leucine, phenylalanine, tyrosine, tryptophan, and methionine. Hydrophilic or polar amino acids typically reside on the exterior or surface of proteins, which have a significant degree of solvent accessibility. Thus at variable surface positions it may be beneficial to consider only or mostly polar amino acids such as alanine, serine, threonine, aspartic acid, asparagine, glutamine, glutamic acid, arginine, lysine and histidine. Some positions are partly exposed and partly buried, and are not clearly protein core or surface positions, in a sense serving as boundary residues between core and surface residues. Thus at such variable boundary positions it may be beneficial to consider both nonpolar and polar amino acids such as alanine, serine, threonine, aspartic acid, asparagine, glutamine, glutamic acid, arginine, lysine histidine, valine, isoleucine, leucine, phenylalanine, tyrosine, tryptophan, and methionine. Determination of the degree of solvent exposure at variable positions may be by subjective evaluation or visual inspection of the template structure by one skilled in the art of protein structural biology, or by using a variety of algorithms that are known in the art. Selection of amino acid types to be considered at variable positions may be aided or determined wholly by computational methods, such as calculation of solvent accessible surface area, or using algorithms that assess the orientation of the C□-C□ vectors relative to a solvent accessible surface, as outlined in US 6,188,965; 6,269,312; US 6,403,312; USSN 09/782,004; USSN 09/927,790; USSN 10/218,102; PCT WO 98/07254; PCT WO 01/40091; and PCT WO 02/25588. In one embodiment, each variable position may be classified explicitly as a core, surface, or boundary position or a classification substantially similar to core, surface, or boundary.

[141] In an alternate embodiment, selection of the set of amino acids allowed at variable positions may be hypothesis-driven. Hypotheses for which amino acid types should be considered at variable positions may be derived by a subjective evaluation or visual inspection of the template structure by one skilled in the art of protein structural biology. For example, if it is suspected that a hydrogen bonding interaction may be favorable at a variable position, polar residues that have the capacity to form hydrogen bonds may be considered, even if the position is in the core. Likewise, if it is suspected that a hydrophobic packing interaction may be favorable at a variable position, nonpolar residues that have the capacity to form favorable packing interactions may be considered, even if the position is on the surface. Other examples of hypothesis-driven approaches may involve issues of backbone flexibility or protein fold. As is known in the art, certain residues, for example proline, glycine, and cysteine, play important roles in protein structure and stability. Glycine enables greater backbone flexibility than all other amino acids, proline constrains the backbone more than all other

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amino acids, and cysteines may form disulfide bonds. It may therefore be beneficial to include one or more of these amino acid types to achieve a desired design goal. Alternatively, it may be beneficial to exclude one or more of these amino acid types from the list of considered amino acids.

[142] In an alternate embodiment, subsets of amino acids may be chosen to maximize coverage. In this case, additional amino acids with properties similar to that in the template structure may be considered at variable positions. For example, if the residue at a variable position in the template structure is a large hydrophobic residue, additional large hydrophobic amino acids may be considered at that position. Alternatively, subsets of amino acids may be chosen to maximize diversity. In this case, amino acids with properties dissimilar to those in the template structure may be considered at variable positions. For example, if the residue at a variable position in the template is a large hydrophobic residue, amino acids that are small, polar, etc. may be considered.

[143] As is known in the art, some computational screening methods require only the identity of considered amino acids to be determined during design calculations. That is, no information is required concerning the conformations or possible conformations of the amino acid side chains. Other preferred methods utilize a set of discrete side chain conformations, called rotamers, which are considered for each amino acid. Thus, a set of rotamers may be considered at each variable and floated position. Rotamers may be obtained from published rotamer libraries (see for example, Lovel *et al.*, 2000, *Proteins: Structure Function and Genetics* 40:389-408; Dunbrack & Cohen, 1997, *Protein Science* 6:1661-1681; DeMaeyer *et al.*, 1997, *Folding and Design* 2:53-66; Tuffery *et al.*, 1991, *J Biomol Struct Dyn* 8:1267-1289, Ponder & Richards, 1987, *J Mol Biol* 193:775-791). As is known in the art, rotamer libraries may be backbone-independent or backbone-dependent. Rotamers may also be obtained from molecular mechanics or *ab initio* calculations, and using other methods. In a preferred embodiment, a flexible rotamer model is used (see Mendes *et al.*, 1999, *Proteins: Structure, Function, and Genetics* 37:530-543). Similarly, artificially generated rotamers may be used, or augment the set chosen for each amino acid and/or variable position. In one embodiment, at least one conformation that is not low in energy is included in the list of rotamers. In an alternate embodiment, the rotamer of the variable position residue in the template structure is included in the list of rotamers allowed for that variable position. In an alternate embodiment, only the identity of each amino acid considered at variable positions is provided, and no specific conformational states of each amino acid are used during design calculations. That is, use of rotamers is not essential for computational screening.

[144] Experimental information may be used to guide the choice of variable positions and/or the choice of considered amino acids at variable positions. As is known in the art, mutagenesis experiments are often carried out to determine the role of certain residues in protein structure and function, for example, which protein residues play a role in determining stability, or which residues

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make up the interface of a protein-protein interaction. Data obtained from such experiments are useful in the present invention. For example, variable positions for Fc/FcγR affinity enhancement could involve varying all positions at which mutation has been shown to affect binding. Similarly, the results from such an experiment may be used to guide the choice of allowed amino acid types at variable positions. For example, if certain types of amino acid substitutions are found to be favorable, similar types of those amino acids may be considered. In one embodiment, additional amino acids with properties similar to those that were found to be favorable experimentally may be considered at variable positions. For example, if experimental mutation of a variable position at an Fc/FcγR interface to a large hydrophobic residue was found to be favorable, the user may choose to include additional large hydrophobic amino acids at that position in the computational screen. As is known in the art, display and other selection technologies may be coupled with random mutagenesis to generate a list or lists of amino acid substitutions that are favorable for the selected property. Such a list or lists obtained from such experimental work find use in the present invention. For example, positions that are found to be invariable in such an experiment may be excluded as variable positions in computational screening calculations, whereas positions that are found to be more acceptable to mutation or respond favorably to mutation may be chosen as variable positions. Similarly, the results from such experiments may be used to guide the choice of allowed amino acid types at variable positions. For example, if certain types of amino acids arise more frequently in an experimental selection, similar types of those amino acids may be considered. In one embodiment, additional amino acids with properties similar to those that were found to be favorable experimentally may be considered at variable positions. For example, if selected mutations at a variable position that resides at an Fc/FcγR interface are found to be uncharged polar amino acids, the user may choose to include additional uncharged polar amino acids, or perhaps charged polar amino acids, at that position.

[145] Sequence information may also be used to guide choice of variable positions and/or the choice of amino acids considered at variable positions. As is known in the art, some proteins share a common structural scaffold and are homologous in sequence. This information may be used to gain insight into particular positions in the protein family. As is known in the art, sequence alignments are often carried out to determine which protein residues are conserved and which are not conserved. That is to say, by comparing and contrasting alignments of protein sequences, the degree of variability at a position may be observed, and the types of amino acids that occur naturally at positions may be observed. Data obtained from such analyses are useful in the present invention. The benefit of using sequence information to choose variable positions and considered amino acids at variable positions are several fold. For choice of variable positions, the primary advantage of using sequence information is that insight may be gained into which positions are more tolerant and which are less tolerant to mutation. Thus sequence information may aid in ensuring that quality diversity, i.e. mutations that are not deleterious to protein structure, stability, etc., is sampled computationally. The same advantage applies to use of sequence information to select amino acid types considered at variable positions. That is, the set of amino acids that occur in a protein sequence alignment may be

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thought of as being pre-screened by evolution to have a higher chance than random for being compatible with a protein's structure, stability, solubility, function, etc. Thus higher quality diversity is sampled computationally. A second benefit of using sequence information to select amino acid types considered at variable positions is that certain alignments may represent sequences that may be less immunogenic than random sequences. For example, if the amino acids considered at a given variable position are the set of amino acids which occur at that position in an alignment of human protein sequences, those amino acids may be thought of as being pre-screened by nature for generating no or low immune response if the optimized protein is used as a human therapeutic.

[146] The source of the sequences may vary widely, and include one or more of the known databases, including but not limited to the Kabat database (Johnson & Wu, 2001, *Nucleic Acids Res* 29:205-206; Johnson & Wu, 2000, *Nucleic Acids Res* 28:214-218), the IMGT database (IMGT, the international ImMunoGeneTics information system®; Lefranc *et al.*, 1999, *Nucleic Acids Res* 27:209-212; Ruiz *et al.*, 2000 *Nucleic Acids Re.* 28:219-221; Lefranc *et al.*, 2001, *Nucleic Acids Res* 29:207-209; Lefranc *et al.*, 2003, *Nucleic Acids Res* 31:307-310), and VBASE, SwissProt, GenBank and Entrez, and EMBL Nucleotide Sequence Database. Protein sequence information can be obtained, compiled, and/or generated from sequence alignments of naturally occurring proteins from any organism, including but not limited to mammals. Protein sequence information can be obtained from a database that is compiled privately. There are numerous sequence-based alignment programs and methods known in the art, and all of these find use in the present invention for generation of sequence alignments of proteins that comprise Fc and Fc ligands.

[147] Once alignments are made, sequence information can be used to guide choice of variable positions. Such sequence information can relate the variability, natural or otherwise, of a given position. Variability herein should be distinguished from variable position. Variability refers to the degree to which a given position in a sequence alignment shows variation in the types of amino acids that occur there. Variable position, to reiterate, is a position chosen by the user to vary in amino acid identity during a computational screening calculation. Variability may be determined qualitatively by one skilled in the art of bioinformatics. There are also methods known in the art to quantitatively determine variability that may find use in the present invention. The most preferred embodiment measures Information Entropy or Shannon Entropy. Variable positions can be chosen based on sequence information obtained from closely related protein sequences, or sequences that are less closely related.

[148] The use of sequence information to choose variable positions finds broad use in the present invention. For example, if an Fc/FcγR interface position in the template structure is tryptophan, and tryptophan is observed at that position in greater than 90% of the sequences in an alignment, it may be beneficial to leave that position fixed. In contrast, if another interface position is found to have a

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greater level of variability, for example if five different amino acids are observed at that position with frequencies of approximately 20% each, that position may be chosen as a variable position. In another embodiment, visual inspection of aligned protein sequences may substitute for or aid visual inspection of a protein structure. Sequence information can also be used to guide the choice of amino acids considered at variable positions. Such sequence information can relate to how frequently an amino acid, amino acids, or amino acid types (for example polar or nonpolar, charged or uncharged) occur, naturally or otherwise, at a given position. In one embodiment, the set of amino acids considered at a variable position may comprise the set of amino acids that is observed at that position in the alignment. Thus, the position-specific alignment information is used directly to generate the list of considered amino acids at a variable position in a computational screening calculation. Such a strategy is well known in the art; see for example Lehmann & Wyss, 2001, *Curr Opin Biotechnol* 12(4):371-5; Lehmann *et al.*, 2000, *Biochim Biophys Acta* 1543(2):408-415; Rath & Davidson, 2000, *Protein Sci*, 9(12):2457-69; Lehmann *et al.*, 2000, *Protein Eng* 13(1):49-57; Desjarlais & Berg, 1993, *Proc Natl Acad Sci USA* 90(6):2256-60; Desjarlais & Berg, 1992, *Proteins* 12(2):101-4; Henikoff & Henikoff, 2000, *Adv Protein Chem* 54:73-97; Henikoff & Henikoff, 1994, *J Mol Biol* 243(4):574-8. In an alternate embodiment, the set of amino acids considered at a variable position or positions may comprise a set of amino acids that is observed most frequently in the alignment. Thus, a certain criteria is applied to determine whether the frequency of an amino acid or amino acid type warrants its inclusion in the set of amino acids that are considered at a variable position. As is known in the art, sequence alignments may be analyzed using statistical methods to calculate the sequence diversity at any position in the alignment and the occurrence frequency or probability of each amino acid at a position. Such data may then be used to determine which amino acids types to consider. In the simplest embodiment, these occurrence frequencies are calculated by counting the number of times an amino acid is observed at an alignment position, then dividing by the total number of sequences in the alignment. In other embodiments, the contribution of each sequence, position or amino acid to the counting procedure is weighted by a variety of possible mechanisms. In a preferred embodiment, the contribution of each aligned sequence to the frequency statistics is weighted according to its diversity weighting relative to other sequences in the alignment. A common strategy for accomplishing this is the sequence weighting system recommended by Henikoff and Henikoff (Henikoff & Henikoff, 2000, *Adv Protein Chem* 54:73-97; Henikoff & Henikoff, 1994, *J Mol Biol* 243:574-8. In a preferred embodiment, the contribution of each sequence to the statistics is dependent on its extent of similarity to the target sequence, i.e. the template structure used, such that sequences with higher similarity to the target sequence are weighted more highly. Examples of similarity measures include, but are not limited to, sequence identity, BLOSUM similarity score, PAM matrix similarity score, and BLAST score. In an alternate embodiment, the contribution of each sequence to the statistics is dependent on its known physical or functional properties. These properties include, but are not limited to, thermal and chemical stability, contribution to activity, and solubility. For example, when optimizing aglycosylated Fc for solubility, those sequences in an alignment that are known to be most soluble (for example see Ewert *et al.*, 2003, *J Mol Biol* 325:531-553), will contribute more heavily to the calculated frequencies.

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[149] Regardless of what criteria are applied for choosing the set of amino acids in a sequence alignment to be considered at variable positions, use of sequence information to choose considered amino acids finds broad use in the present invention. For example, to optimize Fc solubility by replacing exposed nonpolar surface residues, considered amino acids may be chosen as the set of amino acids, or a subset of those amino acids which meet some criteria, that are observed at that position in an alignment of protein sequences. As another example, one or more amino acids may be added or subtracted subjectively from a list of amino acids derived from a sequence alignment in order to maximize coverage. For example, additional amino acids with properties similar to those that are found in a sequence alignment may be considered at variable positions. For example, if an Fc position that is known to or hypothesized to bind an FcγR is observed to have uncharged polar amino acids in a sequence alignment, the user may choose to include additional uncharged polar amino acids in a computational screening calculation, or perhaps charged polar amino acids, at that position.

[150] In one embodiment, sequence alignment information is combined with energy calculation, as discussed below. For example, pseudo energies can be derived from sequence information to generate a scoring function. The use of a sequence-based scoring function may assist in significantly reducing the complexity of a calculation. However, as is appreciated by those skilled in the art, the use of a sequence-based scoring function alone may be inadequate because sequence information can often indicate misleading correlations between mutations that may in reality be structurally conflicting. Thus, in a preferred embodiment, a structure-based method of energy calculation is used, either alone or in combination with a sequence-based scoring function. That is, preferred embodiments do not rely on sequence alignment information alone as the analysis step.

[151] Energy calculation refers to the process by which amino acid modifications are scored. The energies of interaction are measured by one or more scoring functions. A variety of scoring functions find use in the present invention for calculating energies. Scoring functions may include any number of potentials, herein referred to as the energy terms of a scoring function, including but not limited to a van der Waals potential, a hydrogen bond potential, an atomic solvation potential or other solvation models, a secondary structure propensity potential, an electrostatic potential, a torsional potential, and an entropy potential. At least one energy term is used to score each variable or floated position, although the energy terms may differ depending on the position, considered amino acids, and other considerations. In one embodiment, a scoring function using one energy term is used. In the most preferred embodiment, energies are calculated using a scoring function that contains more than one energy term, for example describing van der Waals, solvation, electrostatic, and hydrogen bond interactions, and combinations thereof. In additional embodiments, additional energy terms include but are not limited to entropic terms, torsional energies, and knowledge-based energies.

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[152] A variety of scoring functions are described in US 6,188,965; US 6,269,312; US 6,403,312; USSN 09/782,004; USSN 09/927,790; USSN 09/877,695; USSN 10/071,859, USSN 10/218,102; PCT WO 98/07254; PCT WO 01/40091; and PCT WO 02/25588. As will be appreciated by those skilled in the art, scoring functions need not be limited to physico-chemical energy terms. For example, knowledge-based potentials may find use in the computational screening methodology of the present invention. Such knowledge-based potentials may be derived from protein sequence and/or structure statistics including but not limited to threading potentials, reference energies, pseudo energies, homology-based energies, and sequence biases derived from sequence alignments. In a preferred embodiment, a scoring function is modified to include models for immunogenicity, such as functions derived from data on binding of peptides to MHC (Major Htocompatability Complex), that may be used to identify potentially immunogenic sequences (see for example USSN 09/903,378; USSN 10/039,170; USSN 60/222,697; USSN 10/339788; PCT WO 01/21823; and PCT WO 02/00165). In one embodiment, sequence alignment information can be used to score amino acid substitutions. For example, comparison of protein sequences, regardless of whether the source of said proteins is human, monkey, mouse, or otherwise, may be used to suggest or score amino acid mutations in the computational screening methodology of the present invention. In one embodiment, as is known in the art, one or more scoring functions may be optimized or "trained" during the computational analysis, and then the analysis re-run using the optimized system. Such altered scoring functions may be obtained for example, by training a scoring function using experimental data. As will be appreciated by those skilled in the art, a number of force fields, which are comprised of one or more energy terms, may serve as scoring functions. Force fields include but are not limited to *ab initio* or quantum mechanical force fields, semi-empirical force fields, and molecular mechanics force fields. Scoring functions that are knowledge-based or that use statistical methods may find use in the present invention. These methods may be used to assess the match between a sequence and a three-dimensional protein structure, and hence may be used to score amino acid substitutions for fidelity to the protein structure. In one embodiment, molecular dynamics calculations may be used to computationally screen sequences by individually calculating mutant sequence scores.

[153] There are a variety of ways to represent amino acids in order to enable efficient energy calculation. In a preferred embodiment, considered amino acids are represented as rotamers, as described previously, and the energy (or score) of interaction of each possible rotamer at each variable and floated position with the other variable and floated rotamers, with fixed position residues, and with the backbone structure and any non-protein atoms, is calculated. In a preferred embodiment, two sets of interaction energies are calculated for each side chain rotamer at every variable and floated position: the interaction energy between the rotamer and the fixed atoms (the "singles" energy), and the interaction energy between the variable and floated positions rotamer and all other possible rotamers at every other variable and floated position (the "doubles" energy). In an alternate embodiment, singles and doubles energies are calculated for fixed positions as well as for variable and floated positions. In an alternate embodiment, considered amino acids are not represented as rotamers.

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[154] An important component of computational screening is the identification of one or more sequences that have a favorable score, i.e. are low in energy. Determining a set of low energy sequences from an extremely large number of possibilities is nontrivial, and to solve this problem a combinatorial optimization algorithm is employed. The need for a combinatorial optimization algorithm is illustrated by examining the number of possibilities that are considered in a typical computational screening calculation. The discrete nature of rotamer sets allows a simple calculation of the number of possible rotameric sequences for a given design problem. A backbone of length n with m possible rotamers per position will have m^n possible rotamer sequences, a number that grows exponentially with sequence length. For very simple calculations, it is possible to examine each possible sequence in order to identify the optimal sequence and/or one or more favorable sequences. However, for a typical design problem, the number of possible sequences (up to 10^{80} or more) is sufficiently large that examination of each possible sequence is intractable. A variety of combinatorial optimization algorithms may then be used to identify the optimum sequence and/or one or more favorable sequences. Combinatorial optimization algorithms may be divided into two classes: (1) those that are guaranteed to return the global minimum energy configuration if they converge, and (2) those that are not guaranteed to return the global minimum energy configuration, but which will always return a solution. Examples of the first class of algorithms include but are not limited to Dead-End Elimination (DEE) and Branch & Bound (B&B) (including Branch and Terminate) (Gordon & Mayo, 1999, *Structure Fold Des* 7:1089-98). Examples of the second class of algorithms include, but are not limited to, Monte Carlo (MC), self-consistent mean field (SCMF), Boltzmann sampling (Metropolis *et al.*, 1953, *J Chem Phys* 21:1087), simulated annealing (Kirkpatrick *et al.*, 1983, *Science*, 220:671-680), genetic algorithm (GA), and Fast and Accurate Side-Chain Topology and Energy Refinement (FASTER) (Desmet, et al., 2002, Proteins, 48:31-43). A combinatorial optimization algorithm may be used alone or in conjunction with another combinatorial optimization algorithm.

[155] In one embodiment of the present invention, the strategy for applying a combinatorial optimization algorithm is to find the global minimum energy configuration. In an alternate embodiment, the strategy is to find one or more low energy or favorable sequences. In an alternate embodiment, the strategy is to find the global minimum energy configuration and then find one or more low energy or favorable sequences. For example, as outlined in USSN 6,269,312, preferred embodiments utilize a Dead End Elimination (DEE) step and a Monte Carlo step. In other embodiments, tabu search algorithms are used or combined with DEE and/or Monte Carlo, among other search methods (see Modern Heuristic Search Methods, edited by V.J. Rayward-Smith *et al.*, 1996, John Wiley & Sons Ltd.; USSN 10/218,102; and PCT WO 02/25588). In another preferred embodiment, a genetic algorithm may be used; see for example USSN 09/877,695 and USSN 10/071,859. As another example, as is more fully described in US 6,188,965; US 6,269,312; US 6,403,312; USSN 09/782,004; USSN 09/927,790; USSN 10/218,102; PCT WO 98/07254; PCT WO 01/40091; and PCT WO 02/25588, the global optimum may be reached, and then further

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computational processing may occur, which generates additional optimized sequences. In the simplest embodiment, design calculations are not combinatorial. That is, energy calculations are used to evaluate amino acid substitutions individually at single variable positions. For other calculations it is preferred to evaluate amino acid substitutions at more than one variable position. In a preferred embodiment, all possible interaction energies are calculated prior to combinatorial optimization. In an alternatively preferred embodiment, energies may be calculated as needed during combinatorial optimization.

Library generation

[156] The present invention provides methods for generating libraries that may subsequently be screened experimentally to single out optimized Fc variants. By "library" as used herein is meant a set of one or more Fc variants. Library may refer to the set of variants in any form. In one embodiment, the library is a list of nucleic acid or amino acid sequences, or a list of nucleic acid or amino acid substitutions at variable positions. For example, the examples used to illustrate the present invention below provide libraries as amino acid substitutions at variable positions. In one embodiment, a library is a list of at least one sequence that are Fc variants optimized for a desired property. For example see, Filikov *et al.*, 2002, *Protein Sci* 11:1452-1461 and Luo *et al.*, 2002, *Protein Sci* 11:1218-1226. In an alternate embodiment, a library may be defined as a combinatorial list, meaning that a list of amino acid substitutions is generated for each variable position, with the implication that each substitution is to be combined with all other designed substitutions at all other variable positions. In this case, expansion of the combination of all possibilities at all variable positions results in a large explicitly defined library. A library may refer to a physical composition of polypeptides that comprise the Fc region or some domain or fragment of the Fc region. Thus a library may refer to a physical composition of antibodies or Fc fusions, either in purified or unpurified form. A library may refer to a physical composition of nucleic acids that encode the library sequences. Said nucleic acids may be the genes encoding the library members, the genes encoding the library members with any operably linked nucleic acids, or expression vectors encoding the library members together with any other operably linked regulatory sequences, selectable markers, fusion constructs, and/or other elements. For example, the library may be a set of mammalian expression vectors that encode Fc library members, the protein products of which may be subsequently expressed, purified, and screened experimentally. As another example, the library may be a display library. Such a library could, for example, comprise a set of expression vectors that encode library members operably linked to some fusion partner that enables phage display, ribosome display, yeast display, bacterial surface display, and the like.

[157] The library may be generated using the output sequence or sequences from computational screening. As discussed above, computationally generated libraries are significantly enriched in stable, properly folded, and functional sequences relative to randomly generated libraries. As a result, computational screening increases the chances of identifying proteins that are optimized for the

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design goal. The set of sequences in a library is generally, but not always, significantly different from the parent sequence, although in some cases the library preferably contains the parent sequence. As is known in the art, there are a variety of ways that a library may be derived from the output of computational screening calculations. For example, methods of library generation described in US 6,403,312; USSN 09/782,004; USSN 09/927,790; USSN 10/218,102; PCT WO 01/40091; and PCT WO 02/25588 find use in the present invention. In one embodiment, sequences scoring within a certain range of the global optimum sequence may be included in the library. For example, all sequences within 10 kcal/mol of the lowest energy sequence could be used as the library. In an alternate embodiment, sequences scoring within a certain range of one or more local minima sequences may be used. In a preferred embodiment, the library sequences are obtained from a filtered set. Such a list or set may be generated by a variety of methods, as is known in the art, for example using an algorithm such as Monte Carlo, B&B, or SCMF. For example, the top 10^3 or the top 10^5 sequences in the filtered set may comprise the library. Alternatively, the total number of sequences defined by the combination of all mutations may be used as a cutoff criterion for the library. Preferred values for the total number of recombined sequences range from 10 to 10^{20}, particularly preferred values range from 100 to 10^9. Alternatively, a cutoff may be enforced when a predetermined number of mutations per position is reached. In some embodiments, sequences that do not make the cutoff are included in the library. This may be desirable in some situations, for instance to evaluate the approach to library generation, to provide controls or comparisons, or to sample additional sequence space. For example, the parent sequence may be included in the library, even if it does not make the cutoff.

[158] Clustering algorithms may be useful for classifying sequences derived by computational screening methods into representative groups. For example, the methods of clustering and their application described in USSN 10/218,102 and PCT WO 02/25588, find use in the present invention. Representative groups may be defined, for example, by similarity. Measures of similarity include, but are not limited to sequence similarity and energetic similarity. Thus the output sequences from computational screening may be clustered around local minima, referred to herein as clustered sets of sequences. For example, sets of sequences that are close in sequence space may be distinguished from other sets. In one embodiment, coverage within one or a subset of clustered sets may be maximized by including in the library some, most, or all of the sequences that make up one or more clustered sets of sequences. For example, it may be advantageous to maximize coverage within the one, two, or three lowest energy clustered sets by including the majority of sequences within these sets in the library. In an alternate embodiment, diversity across clustered sets of sequences may be sampled by including within a library only a subset of sequences within each clustered set. For example, all or most of the clustered sets could be broadly sampled by including the lowest energy sequence from each clustered set in the library.

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[159] Sequence information may be used to guide or filter computationally screening results for generation of a library. As discussed, by comparing and contrasting alignments of protein sequences, the degree of variability at a position and the types of amino acids which occur naturally at that position may be observed. Data obtained from such analyses are useful in the present invention. The benefits of using sequence information have been discussed, and those benefits apply equally to use of sequence information to guide library generation. The set of amino acids that occur in a sequence alignment may be thought of as being pre-screened by evolution to have a higher chance than random at being compatible with a protein's structure, stability, solubility, function, and immunogenicity. The variety of sequence sources, as well as the methods for generating sequence alignments that have been discussed, find use in the application of sequence information to guiding library generation. Likewise, as discussed above, various criteria may be applied to determine the importance or weight of certain residues in an alignment. These methods also find use in the application of sequence information to guide library generation. Using sequence information to guide library generation from the results of computational screening finds broad use in the present invention. In one embodiment, sequence information is used to filter sequences from computational screening output. That is to say, some substitutions are subtracted from the computational output to generate the library. For example the resulting output of a computational screening calculation or calculations may be filtered so that the library includes only those amino acids, or a subset of those amino acids that meet some criteria, for example that are observed at that position in an alignment of sequences. In an alternate embodiment, sequence information is used to add sequences to the computational screening output. That is to say, sequence information is used to guide the choice of additional amino acids that are added to the computational output to generate the library. For example, the output set of amino acids for a given position from a computational screening calculation may be augmented to include one or more amino acids that are observed at that position in an alignment of protein sequences. In an alternate embodiment, based on sequence alignment information, one or more amino acids may be added to or subtracted from the computational screening sequence output in order to maximize coverage or diversity. For example, additional amino acids with properties similar to those that are found in a sequence alignment may be added to the library. For example, if a position is observed to have uncharged polar amino acids in a sequence alignment, additional uncharged polar amino acids may be included in the library at that position.

[160] Libraries may be processed further to generate subsequent libraries. In this way, the output from a computational screening calculation or calculations may be thought of as a primary library. This primary library may be combined with other primary libraries from other calculations or other libraries, processed using subsequent calculations, sequence information, or other analyses, or processed experimentally to generate a subsequent library, herein referred to as a secondary library. As will be appreciated from this description, the use of sequence information to guide or filter libraries, discussed above, is itself one method of generating secondary libraries from primary libraries. Generation of secondary libraries gives the user greater control of the parameters within a library.

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This enables more efficient experimental screening, and may allow feedback from experimental results to be interpreted more easily, providing a more efficient design/experimentation cycle.

[161] There are a wide variety of methods to generate secondary libraries from primary libraries. For example, USSN 10/218,102 and PCT WO 02/25588, describes methods for secondary library generation that find use in the present invention. Typically some selection step occurs in which a primary library is processed in some way. For example, in one embodiment a selection step occurs wherein some set of primary sequences are chosen to form the secondary library. In an alternate embodiment, a selection step is a computational step, again generally including a selection step, wherein some subset of the primary library is chosen and then subjected to further computational analysis, including both further computational screening as well as techniques such as "*in silico*" shuffling or recombination (see, for example US 5,830,721; US 5,811,238; US 5,605,793; and US 5,837,458, error-prone PCR, for example using modified nucleotides; known mutagenesis techniques including the use of multi-cassettes; and DNA shuffling (Crameri *et al.*, 1998, *Nature* 391:288-291; Coco *et al.*, 2001, *Nat Biotechnol* 19:354-9; Coco *et al.*, 2002, *Nat Biotechnol*, 20:1246-50), heterogeneous DNA samples (US 5,939,250); ITCHY (Ostermeier *et al.*, 1999, *Nat Biotechnol* 17:1205-1209); StEP (Zhao *et al.*, 1998, *Nat Biotechnol* 16:258-261), GSSM (US 6,171,820 and US 5,965,408); *in vivo* homologous recombination, ligase assisted gene assembly, end-complementary PCR, profusion (Roberts & Szostak, 1997, *Proc Natl Acad Sci USA* 94:12297-12302); yeast/bacteria surface display (Lu *et al.*, 1995, *Biotechnology* 13:366-372); Seed & Aruffo, 1987, *Proc Natl Acad Sci USA* 84(10):3365-3369; Boder & Wittrup, 1997, *Nat Biotechnol* 15:553-557). In an alternate embodiment, a selection step occurs that is an experimental step, for example any of the library screening steps below, wherein some subset of the primary library is chosen and then recombined experimentally, for example using one of the directed evolution methods discussed below, to form a secondary library. In a preferred embodiment, the primary library is generated and processed as outlined in US 6,403,312.

[162] Generation of secondary and subsequent libraries finds broad use in the present invention. In one embodiment, different primary libraries may be combined to generate a secondary or subsequent library. In another embodiment, secondary libraries may be generated by sampling sequence diversity at highly mutatable or highly conserved positions. The primary library may be analyzed to determine which amino acid positions in the template protein have high mutational frequency, and which positions have low mutational frequency. For example, positions in a protein that show a great deal of mutational diversity in computational screening may be fixed in a subsequent round of design calculations. A filtered set of the same size as the first would now show diversity at positions that were largely conserved in the first library. Alternatively, the secondary library may be generated by varying the amino acids at the positions that have high numbers of mutations, while keeping constant the positions that do not have mutations above a certain frequency.

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[163] This discussion is not meant to constrain generation of libraries subsequent to primary libraries to secondary libraries. As will be appreciated, primary and secondary libraries may be processed further to generate tertiary libraries, quaternary libraries, and so on. In this way, library generation is an iterative process. For example, tertiary libraries may be constructed using a variety of additional steps applied to one or more secondary libraries; for example, further computational processing may occur, secondary libraries may be recombined, or subsets of different secondary libraries may be combined. In a preferred embodiment, a tertiary library may be generated by combining secondary libraries. For example, primary and/or secondary libraries that analyzed different parts of a protein may be combined to generate a tertiary library that treats the combined parts of the protein. In an alternate embodiment, the variants from a primary library may be combined with the variants from another primary library to provide a combined tertiary library at lower computational cost than creating a very long filtered set. These combinations may be used, for example, to analyze large proteins, especially large multi-domain proteins, of which Fc is an example. Thus the above description of secondary library generation applies to generating any library subsequent to a primary library, the end result being a final library that may screened experimentally to obtain protein variants optimized for a design goal. These examples are not meant to constrain generation of secondary libraries to any particular application or theory of operation for the present invention. Rather, these examples are meant to illustrate that generation of secondary libraries, and subsequent libraries such as tertiary libraries and so on, is broadly useful in computational screening methodology for library generation.

Experimental Production and Screening

[164] The present invention provides methods for producing and screening libraries of Fc variants. The described methods are not meant to constrain the present invention to any particular application or theory of operation. Rather, the provided methods are meant to illustrate generally that one or more Fc variants or one or more libraries of Fc variants may be produced and screened experimentally to obtain optimized Fc variants. Fc variants may be produced and screened in any context, whether as an Fc region as precisely defined herein, a domain or fragment thereof, or a larger polypeptide that comprises Fc such as an antibody or Fc fusion. General methods for antibody molecular biology, expression, purification, and screening are described in Antibody Engineering, edited by Duebel & Kontermann, Springer-Verlag, Heidelberg, 2001; and Hayhurst & Georgiou, 2001, *Curr Opin Chem Biol* 5:683-689; Maynard & Georgiou, 2000, *Annu Rev Biomed Eng* 2:339-76.

[165] In one embodiment of the present invention, the library sequences are used to create nucleic acids that encode the member sequences, and that may then be cloned into host cells, expressed and assayed, if desired. Thus, nucleic acids, and particularly DNA, may be made that encode each member protein sequence. These practices are carried out using well-known procedures. For example, a variety of methods that may find use in the present invention are described in Molecular Cloning - A Laboratory Manual, 3rd Ed. (Maniatis, Cold Spring Harbor Laboratory Press, New York,

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2001), and Current Protocols in Molecular Biology (John Wiley & Sons). As will be appreciated by those skilled in the art, the generation of exact sequences for a library comprising a large number of sequences is potentially expensive and time consuming. Accordingly, there are a variety of techniques that may be used to efficiently generate libraries of the present invention. Such methods that may find use in the present invention are described or referenced in US 6,403,312; USSN 09/782,004; USSN 09/927,790; USSN 10/218,102; PCT WO 01/40091; and PCT WO 02/25588. Such methods include but are not limited to gene assembly methods, PCR-based method and methods which use variations of PCR, ligase chain reaction-based methods, pooled oligo methods such as those used in synthetic shuffling, error-prone amplification methods and methods which use oligos with random mutations, classical site-directed mutagenesis methods, cassette mutagenesis, and other amplification and gene synthesis methods. As is known in the art, there are a variety of commercially available kits and methods for gene assembly, mutagenesis, vector subcloning, and the like, and such commercial products find use in the present invention for generating nucleic acids that encode Fc variant members of a library.

[166] The Fc variants of the present invention may be produced by culturing a host cell transformed with nucleic acid, preferably an expression vector, containing nucleic acid encoding the Fc variants, under the appropriate conditions to induce or cause expression of the protein. The conditions appropriate for expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. A wide variety of appropriate host cells may be used, including but not limited to mammalian cells, bacteria, insect cells, and yeast. For example, a variety of cell lines that may find use in the present invention are described in the ATCC® cell line catalog, available from the American Type Culture Collection.

[167] In a preferred embodiment, the Fc variants are expressed in mammalian expression systems, including systems in which the expression constructs are introduced into the mammalian cells using virus such as retrovirus or adenovirus. Any mammalian cells may be used, with human, mouse, rat, hamster, and primate cells being particularly preferred. Suitable cells also include known research cells, including but not limited to Jurkat T cells, NIH3T3, CHO, COS, and 293 cells. In an alternately preferred embodiment, library proteins are expressed in bacterial cells. Bacterial expression systems are well known in the art, and include *Escherichia coli* (*E. coli*), *Bacillus subtilis*, *Streptococcus cremoris*, and *Streptococcus lividans*. In alternate embodiments, Fc variants are produced in insect cells or yeast cells. In an alternate embodiment, Fc variants are expressed *in vitro* using cell free translation systems. In vitro translation systems derived from both prokaryotic (e.g. *E. coli*) and eukaryotic (e.g. wheat germ, rabbit reticulocytes) cells are available and may be chosen based on the expression levels and functional properties of the protein of interest. For example, as appreciated by those skilled in the art, *in vitro* translation is required for some display technologies, for example ribosome display. In addition, the Fc variants may be produced by chemical synthesis methods.

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[168] The nucleic acids that encode the Fc variants of the present invention may be incorporated into an expression vector in order to express the protein. A variety of expression vectors may be utilized for protein expression. Expression vectors may comprise self-replicating extra-chromosomal vectors or vectors which integrate into a host genome. Expression vectors are constructed to be compatible with the host cell type. Thus expression vectors which find use in the present invention include but are not limited to those which enable protein expression in mammalian cells, bacteria, insect cells, yeast, and in *in vitro* systems. As is known in the art, a variety of expression vectors are available, commercially or otherwise, that may find use in the present invention for expressing Fc variant proteins.

[169] Expression vectors typically comprise a protein operably linked with control or regulatory sequences, selectable markers, any fusion partners, and/or additional elements. By "operably linked" herein is meant that the nucleic acid is placed into a functional relationship with another nucleic acid sequence. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the Fc variant, and are typically appropriate to the host cell used to express the protein. In general, the transcriptional and translational regulatory sequences may include promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. As is also known in the art, expression vectors typically contain a selection gene or marker to allow the selection of transformed host cells containing the expression vector. Selection genes are well known in the art and will vary with the host cell used.

[170] Fc variants may be operably linked to a fusion partner to enable targeting of the expressed protein, purification, screening, display, and the like. Fusion partners may be linked to the Fc variant sequence via a linker sequences. The linker sequence will generally comprise a small number of amino acids, typically less than ten, although longer linkers may also be used. Typically, linker sequences are selected to be flexible and resistant to degradation. As will be appreciated by those skilled in the art, any of a wide variety of sequences may be used as linkers. For example, a common linker sequence comprises the amino acid sequence GGGGS. A fusion partner may be a targeting or signal sequence that directs Fc variant protein and any associated fusion partners to a desired cellular location or to the extracellular media. As is known in the art, certain signaling sequences may target a protein to be either secreted into the growth media, or into the periplasmic space, located between the inner and outer membrane of the cell. A fusion partner may also be a sequence that encodes a peptide or protein that enables purification and/or screening. Such fusion partners include but are not limited to polyhistidine tags (His-tags) (for example H_6 and H_{10} or other tags for use with Immobilized Metal Affinity Chromatography (IMAC) systems (e.g. Ni^{+2} affinity columns)), GST fusions, MBP fusions, Strep-tag, the BSP biotinylation target sequence of the bacterial enzyme BirA, and epitope tags which are targeted by antibodies (for example c-myc tags, flag-tags, and the like). As will be appreciated by those skilled in the art, such tags may be useful for purification, for screening, or both.

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For example, an Fc variant may be purified using a His-tag by immobilizing it to a Ni^{+2} affinity column, and then after purification the same His-tag may be used to immobilize the antibody to a Ni^{+2} coated plate to perform an ELISA or other binding assay (as described below). A fusion partner may enable the use of a selection method to screen Fc variants (see below). Fusion partners that enable a variety of selection methods are well-known in the art, and all of these find use in the present invention. For example, by fusing the members of an Fc variant library to the gene III protein, phage display can be employed (Kay *et al.*, Phage display of peptides and proteins: a laboratory manual, Academic Press, San Diego, CA, 1996; Lowman *et al.*, 1991, *Biochemistry* 30:10832-10838; Smith, 1985, *Science* 228:1315-1317). Fusion partners may enable Fc variants to be labeled. Alternatively, a fusion partner may bind to a specific sequence on the expression vector, enabling the fusion partner and associated Fc variant to be linked covalently or noncovalently with the nucleic acid that encodes them. For example, USSN 09/642,574; USSN 10/080,376; USSN 09/792,630; USSN 10/023,208; USSN 09/792,626; USSN 10/082,671; USSN 09/953,351; USSN 10/097,100; USSN 60/366,658; PCT WO 00/22906; PCT WO 01/49058; PCT WO 02/04852; PCT WO 02/04853; PCT WO 02/08023; PCT WO 01/28702; and PCT WO 02/07466 describe such a fusion partner and technique that may find use in the present invention.

[171] The methods of introducing exogenous nucleic acid into host cells are well known in the art, and will vary with the host cell used. Techniques include but are not limited to dextran-mediated transfection, calcium phosphate precipitation, calcium chloride treatment, polybrene mediated transfection, protoplast fusion, electroporation, viral or phage infection, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In the case of mammalian cells, transfection may be either transient or stable.

[172] In a preferred embodiment, Fc variant proteins are purified or isolated after expression. Proteins may be isolated or purified in a variety of ways known to those skilled in the art. Standard purification methods include chromatographic techniques, including ion exchange, hydrophobic interaction, affinity, sizing or gel filtration, and reversed-phase, carried out at atmospheric pressure or at high pressure using systems such as FPLC and HPLC. Purification methods also include electrophoretic, immunological, precipitation, dialysis, and chromatofocusing techniques. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. As is well known in the art, a variety of natural proteins bind Fc and antibodies, and these proteins can find use in the present invention for purification of Fc variants. For example, the bacterial proteins A and G bind to the Fc region. Likewise, the bacterial protein L binds to the Fab region of some antibodies, as of course does the antibody's target antigen. Purification can often be enabled by a particular fusion partner. For example, Fc variant proteins may be purified using glutathione resin if a GST fusion is employed, Ni^{+2} affinity chromatography if a His-tag is employed, or immobilized anti-flag antibody if a flag-tag is used. For general guidance in suitable purification techniques, see Protein Purification: Principles and Practice, 3rd Ed., Scopes, Springer-Verlag, NY, 1994. The degree of

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purification necessary will vary depending on the screen or use of the Fc variants. In some instances no purification is necessary. For example in one embodiment, if the Fc variants are secreted, screening may take place directly from the media. As is well known in the art, some methods of selection do not involve purification of proteins. Thus, for example, if a library of Fc variants is made into a phage display library, protein purification may not be performed.

[173] Fc variants may be screened using a variety of methods, including but not limited to those that use *in vitro* assays, *in vivo* and cell-based assays, and selection technologies. Automation and high-throughput screening technologies may be utilized in the screening procedures. Screening may employ the use of a fusion partner or label. The use of fusion partners has been discussed above. By "labeled" herein is meant that the Fc variants of the invention have one or more elements, isotopes, or chemical compounds attached to enable the detection in a screen. In general, labels fall into three classes: a) immune labels, which may be an epitope incorporated as a fusion partner that is recognized by an antibody, b) isotopic labels, which may be radioactive or heavy isotopes, and c) small molecule labels, which may include fluorescent and colorimetric dyes, or molecules such as biotin that enable other labeling methods. Labels may be incorporated into the compound at any position and may be incorporated *in vitro* or *in vivo* during protein expression.

[174] In a preferred embodiment, the functional and/or biophysical properties of Fc variants are screened in an *in vitro* assay. *In vitro* assays may allow a broad dynamic range for screening properties of interest. Properties of Fc variants that may be screened include but are not limited to stability, solubility, and affinity for Fc ligands, for example Fc□Rs. Multiple properties may be screened simultaneously or individually. Proteins may be purified or unpurified, depending on the requirements of the assay. In one embodiment, the screen is a qualitative or quantitative binding assay for binding of Fc variants to a protein or nonprotein molecule that is known or thought to bind the Fc variant. In a preferred embodiment, the screen is a binding assay for measuring binding to the antibody's or Fc fusions' target antigen. In an alternately preferred embodiment, the screen is an assay for binding of Fc variants to an Fc ligand, including but are not limited to the family of Fc□Rs, the neonatal receptor FcRn, the complement protein C1q, and the bacterial proteins A and G. Said Fc ligands may be from any organism, with humans, mice, rats, rabbits, and monkeys preferred. Binding assays can be carried out using a variety of methods known in the art, including but not limited to FRET (Fluorescence Resonance Energy Transfer) and BRET (Bioluminescence Resonance Energy Transfer) -based assays, AlphaScreen™ (Amplified Luminescent Proximity Homogeneous Assay), Scintillation Proximity Assay, ELISA (Enzyme-Linked Immunosorbent Assay), SPR (Surface Plasmon Resonance, also known as BIACORE®), isothermal titration calorimetry, differential scanning calorimetry, gel electrophoresis, and chromatography including gel filtration. These and other methods may take advantage of some fusion partner or label of the Fc variant. Assays may employ a variety of detection methods including but not limited to chromogenic, fluorescent, luminescent, or isotopic labels.

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[175] The biophysical properties of Fc variant proteins, for example stability and solubility, may be screened using a variety of methods known in the art. Protein stability may be determined by measuring the thermodynamic equilibrium between folded and unfolded states. For example, Fc variant proteins of the present invention may be unfolded using chemical denaturant, heat, or pH, and this transition may be monitored using methods including but not limited to circular dichroism spectroscopy, fluorescence spectroscopy, absorbance spectroscopy, NMR spectroscopy, calorimetry, and proteolysis. As will be appreciated by those skilled in the art, the kinetic parameters of the folding and unfolding transitions may also be monitored using these and other techniques. The solubility and overall structural integrity of an Fc variant protein may be quantitatively or qualitatively determined using a wide range of methods that are known in the art. Methods which may find use in the present invention for characterizing the biophysical properties of Fc variant proteins include gel electrophoresis, chromatography such as size exclusion chromatography and reversed-phase high performance liquid chromatography, mass spectrometry, ultraviolet absorbance spectroscopy, fluorescence spectroscopy, circular dichroism spectroscopy, isothermal titration calorimetry, differential scanning calorimetry, analytical ultra-centrifugation, dynamic light scattering, proteolysis, and cross-linking, turbidity measurement, filter retardation assays, immunological assays, fluorescent dye binding assays, protein-staining assays, microscopy, and detection of aggregates via ELISA or other binding assay. Structural analysis employing X-ray crystallographic techniques and NMR spectroscopy may also find use. In one embodiment, stability and/or solubility may be measured by determining the amount of protein solution after some defined period of time. In this assay, the protein may or may not be exposed to some extreme condition, for example elevated temperature, low pH, or the presence of denaturant. Because function typically requires a stable, soluble, and/or well-folded/structured protein, the aforementioned functional and binding assays also provide ways to perform such a measurement. For example, a solution comprising an Fc variant could be assayed for its ability to bind target antigen, then exposed to elevated temperature for one or more defined periods of time, then assayed for antigen binding again. Because unfolded and aggregated protein is not expected to be capable of binding antigen, the amount of activity remaining provides a measure of the Fc variant's stability and solubility.

[176] In a preferred embodiment, the library is screened using one or more cell-based or *in vivo* assays. For such assays, Fc variant proteins, purified or unpurified, are typically added exogenously such that cells are exposed to individual variants or pools of variants belonging to a library. These assays are typically, but not always, based on the function of an antibody or Fc fusion that comprises the Fc variant; that is, the ability of the antibody or Fc fusion to bind a target antigen and mediate some biochemical event, for example effector function, ligand/receptor binding inhibition, apoptosis, and the like. Such assays often involve monitoring the response of cells to antibody or Fc fusion, for example cell survival, cell death, change in cellular morphology, or transcriptional activation such as cellular expression of a natural gene or reporter gene. For example, such assays may measure the

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ability of Fc variants to elicit ADCC, ADCP, or CDC. For some assays additional cells or components, that is in addition to the target cells, may need to be added, for example example serum complement, or effector cells such as peripheral blood monocytes (PBMCs), NK cells, macrophages, and the like. Such additional cells may be from any organism, preferably humans, mice, rat, rabbit, and monkey. Antibodies and Fc fusions may cause apoptosis of certain cell lines expressing the antibody's target antigen, or they may mediate attack on target cells by immune cells which have been added to the assay. Methods for monitoring cell death or viability are known in the art, and include the use of dyes, immunochemical, cytochemical, and radioactive reagents. For example, caspase staining assays may enable apoptosis to be measured, and uptake or release of radioactive substrates or fluorescent dyes such as alamar blue may enable cell growth or activation to be monitored. In a preferred embodiment, the DELFIA® EuTDA-based cytotoxicity assay (Perkin Elmer, MA) is used. Alternatively, dead or damaged target cells may be monitoried by measuring the release of one or more natural intracellular proteins, for example lactate dehydrogenase. Transcriptional activation may also serve as a method for assaying function in cell-based assays. In this case, response may be monitored by assaying for natural genes or proteins which may be upregulated, for example the release of certain interleukins may be measured, or alternatively readout may be via a reporter construct. Cell-based assays may also involve the measure of morphological changes of cells as a response to the presence of an Fc variant. Cell types for such assays may be prokaryotic or eukaryotic, and a variety of cell lines that are known in the art may be employed.

[177] Alternatively, cell-based screens are performed using cells that have been transformed or transfected with nucleic acids encoding the Fc variants. That is, Fc variant proteins are not added exogenously to the cells. For example, in one embodiment, the cell-based screen utilizes cell surface display. A fusion partner can be employed that enables display of Fc variants on the surface of cells (Witrrup, 2001, *Curr Opin Biotechnol*, 12:395-399). Cell surface display methods that may find use in the present invention include but are not limited to display on bacteria (Georgiou *et al.*, 1997, *Nat Biotechnol* 15:29-34; Georgiou *et al.*, 1993, *Trends Biotechnol* 11:6-10; Lee *et al.*, 2000, *Nat Biotechnol* 18:645-648; Jun *et al.*, 1998, *Nat Biotechnol* 16:576-80), yeast (Boder & Wittrup, 2000, *Methods Enzymol* 328:430-44; Boder & Wittrup, 1997, *Nat Biotechnol* 15:553-557), and mammalian cells (Whitehorn *et al.*, 1995, *Bio/technology* 13:1215-1219). In an alternate embodiment, Fc variant proteins are not displayed on the surface of cells, but rather are screened intracellularly or in some other cellular compartment. For example, periplasmic expression and cytometric screening (Chen *et al.*, 2001, *Nat Biotechnol* 19: 537-542), the protein fragment complementation assay (Johnsson & Varshavsky, 1994, *Proc Natl Acad Sci USA* 91:10340-10344.; Pelletier *et al.*, 1998, *Proc Natl Acad Sci USA* 95:12141-12146), and the yeast two hybrid screen (Fields & Song, 1989, Nature 340:245-246) may find use in the present invention. Alternatively, if a polypeptide that comprises the Fc variants, for example an antibody or Fc fusion, imparts some selectable growth advantage to a cell, this property may be used to screen or select for Fc variants.

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[178] As is known in the art, a subset of screening methods are those that select for favorable members of a library. Said methods are herein referred to as "selection methods", and these methods find use in the present invention for screening Fc variant libraries. When libraries are screened using a selection method, only those members of a library that are favorable, that is which meet some selection criteria, are propagated, isolated, and/or observed. As will be appreciated, because only the most fit variants are observed, such methods enable the screening of libraries that are larger than those screenable by methods that assay the fitness of library members individually. Selection is enabled by any method, technique, or fusion partner that links, covalently or noncovalently, the phenotype of an Fc variant with its genotype, that is the function of an Fc variant with the nucleic acid that encodes it. For example the use of phage display as a selection method is enabled by the fusion of library members to the gene III protein. In this way, selection or isolation of variant proteins that meet some criteria, for example binding affinity for an FcγR, also selects for or isolates the nucleic acid that encodes it. Once isolated, the gene or genes encoding Fc variants may then be amplified. This process of isolation and amplification, referred to as panning, may be repeated, allowing favorable Fc variants in the library to be enriched. Nucleic acid sequencing of the attached nucleic acid ultimately allows for gene identification.

[179] A variety of selection methods are known in the art that may find use in the present invention for screening Fc variant libraries. These include but are not limited to phage display (Phage display of peptides and proteins: a laboratory manual, Kay *et al.*, 1996, Academic Press, San Diego, CA, 1996; Lowman *et al.*, 1991, *Biochemistry* 30:10832-10838; Smith, 1985, *Science* 228:1315-1317) and its derivatives such as selective phage infection (Malmborg *et al.*, 1997, J Mol Biol 273:544-551), selectively infective phage (Krebber *et al.*, 1997, *J Mol Biol* 268:619-630), and delayed infectivity panning (Benhar *et al.*, 2000, *J Mol Biol* 301:893-904), cell surface display (Witrrup, 2001, *Curr Opin Biotechnol*, 12:395-399) such as display on bacteria (Georgiou *et al.*, 1997, *Nat Biotechnol* 15:29-34; Georgiou *et al.*, 1993, *Trends Biotechnol* 11:6-10; Lee *et al.*, 2000, *Nat Biotechnol* 18:645-648; Jun *et al.*, 1998, *Nat Biotechnol* 16:576-80), yeast (Boder & Wittrup, 2000, *Methods Enzymol* 328:430-44; Boder & Wittrup, 1997, *Nat Biotechnol* 15:553-557), and mammalian cells (Whitehorn *et al.*, 1995, *Bio/technology* 13:1215-1219), as well as *in vitro* display technologies (Amstutz *et al.*, 2001, *Curr Opin Biotechnol* 12:400-405) such as polysome display (Mattheakis *et al.*, 1994, *Proc Natl Acad Sci USA* 91:9022-9026), ribosome display (Hanes *et al.*, 1997, *Proc Natl Acad Sci USA* 94:4937-4942), mRNA display (Roberts & Szostak, 1997, *Proc Natl Acad Sci USA 94*:12297-12302; Nemoto *et al.*, 1997, *FEBS Lett* 414:405-408), and ribosome-inactivation display system (Zhou *et al.*, 2002, *J Am Chem Soc* 124, 538-543)

[180] Other selection methods that may find use in the present invention include methods that do not rely on display, such as *in vivo* methods including but not limited to periplasmic expression and cytometric screening (Chen *et al.*, 2001, *Nat Biotechnol* 19:537-542), the protein fragment complementation assay (Johnsson & Varshavsky, 1994, *Proc Natl Acad Sci USA* 91:10340-10344;

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Pelletier *et al.*, 1998, *Proc Natl Acad Sci USA* 95:12141-12146), and the yeast two hybrid screen (Fields & Song, 1989, *Nature* 340:245-246) used in selection mode (Visintin *et al.*, 1999, *Proc Natl Acad Sci USA* 96:11723-11728). In an alternate embodiment, selection is enabled by a fusion partner that binds to a specific sequence on the expression vector, thus linking covalently or noncovalently the fusion partner and associated Fc variant library member with the nucleic acid that encodes them. For example, USSN 09/642,574; USSN 10/080,376; USSN 09/792,630; USSN 10/023,208; USSN 09/792,626; USSN 10/082,671; USSN 09/953,351; USSN 10/097,100; USSN 60/366,658; PCT WO 00/22906; PCT WO 01/49058; PCT WO 02/04852; PCT WO 02/04853; PCT WO 02/08023; PCT WO 01/28702; and PCT WO 02/07466 describe such a fusion partner and technique that may find use in the present invention. In an alternative embodiment, *in vivo* selection can occur if expression of a polypeptide that comprises the Fc variant, such as an antibody or Fc fusion, imparts some growth, reproduction, or survival advantage to the cell.

[181] A subset of selection methods referred to as "directed evolution" methods are those that include the mating or breading of favorable sequences during selection, sometimes with the incorporation of new mutations. As will be appreciated by those skilled in the art, directed evolution methods can facilitate identification of the most favorable sequences in a library, and can increase the diversity of sequences that are screened. A variety of directed evolution methods are known in the art that may find use in the present invention for screening Fc variant libraries, including but not limited to DNA shuffling (PCT WO 00/42561 A3; PCT WO 01/70947 A3), exon shuffling (US 6,365,377; Kolkman & Stemmer, 2001, *Nat Biotechnol* 19:423-428), family shuffling (Crameri *et al.*, 1998, *Nature* 391:288-291; US 6,376,246), RACHITT™ (Coco *et al.*, 2001, *Nat Biotechnol* 19:354- 359; PCT WO 02/06469), STEP and random priming of *in vitro* recombination (Zhao *et al.*, 1998, *Nat Biotechnol* 16:258-261; Shao *et al.*, 1998, *Nucleic Acids Res* 26:681-683), exonuclease mediated gene assembly (US 6,352,842; US 6,361,974), Gene Site Saturation Mutagenesis™ (US 6,358,709), Gene Reassembly™ (US 6,358,709), SCRATCHY (Lutz et al., 2001, *Proc Natl Acad Sci USA* 98:11248-11253), DNA fragmentation methods (Kikuchi *et al.*, *Gene* 236:159-167), single-stranded DNA shuffling (Kikuchi *et al.*, 2000, *Gene* 243:133-137), and AMEsystem™ directed evolution protein engineering technology (Applied Molecular Evolution) (US US 5,824,514; US 5,817,483; US 5,814,476; US 5,763,192; US 5,723,323).

[182] The biological properties of the antibodies and Fc fusions that comprise the Fc variants of the present invention may be characterized in cell, tissue, and whole organism experiments. As is know in the art, drugs are often tested in animals, including but not limited to mice, rats, rabbits, dogs, cats, pigs, and monkeys, in order to measure a drug's efficacy for treatment against a disease or disease model, or to measure a drug's pharmacokinetics, toxicity, and other properties. Said animals may be referred to as disease models. Therapeutics are often tested in mice, including but not limited to nude mice, SCID mice, xenograft mice, and transgenic mice (including knockins and knockouts). For example, an antibody or Fc fusion of the present invention that is intended as an anti-cancer

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therapeutic may be tested in a mouse cancer model, for example a xenograft mouse. In this method, a tumor or tumor cell line is grafted onto or injected into a mouse, and subsequently the mouse is treated with the therapeutic to determine the ability of the antibody or Fc fusion to reduce or inhibit cancer growth. Such experimentation may provide meaningful data for determination of the potential of said antibody or Fc fusion to be used as a therapeutic. Any organism, preferably mammals, may be used for testing. For example because of their genetic similarity to humans, monkeys can be suitable therapeutic models, and thus may be used to test the efficacy, toxicity, pharmacokinetics, or other property of the antibodies and Fc fusions of the present invention. Tests of the antibodies and Fc fusions of the present invention in humans are ultimately required for approval as drugs, and thus of course these experiments are contemplated. Thus the antibodies and Fc fusions of the present invention may be tested in humans to determine their therapeutic efficacy, toxicity, pharmacokinetics, and/or other clinical properties.

EXAMPLES

[183] Examples are provided below to illustrate the present invention. These examples are not meant to constrain the present invention to any particular application or theory of operation.

[184] For all positions discussed in the present invention, numbering is according to the EU index as in Kabat (Kabat *et al.*, 1991, *Sequences of Proteins of Immunological Interest,* 5th Ed., United States Public Health Svice, National Institutes of Health, Bethesda). Those skilled in the art of antibodies will appreciate that this convention consists of nonsequential numbering in specific regions of an immunoglobulin sequence, enabling a normalized reference to conserved positions in immunoglobulin families. Accordingly, the positions of any given immunoglobulin as defined by the EU index will not necessarily correspond to its sequential sequence. Figure 3 shows the sequential and EU index numbering schemes for the antibody alemtuzumab in order to illustrate this principal more clearly. It should also be noted that polymorphisms have been observed at a number of Fc positions, including but not limited to Kabat 270, 272, 312, 315, 356, and 358, and thus slight differences between the presented sequence and sequences in the scientific literature may exist.

[185] Example 1. Computational Screening and Design of Fc Libraries

Computational screening calculations were carried out to design optimized Fc variants. Fc variants were computationally screened, constructed, and experimentally investigated over several computation/experimention cycles. For each successive cycle, experimental data provided feedback into the next set of computational screening calculations and library design. All computational screening calculations and library design are presented in Example 1. For each set of calculations, a table is provided that presents the results and provides relevant information and parameters.

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[186] Several different structures of Fc bound bound to the extracellular domain of Fc□Rs served as template structures for the computational screening calculations. Publicly available Fc/FcγR complex structures included pdb accession code 1E4K (Sondermann *et al.*, 2000, *Nature* 406:267-273.), and pdb accession codes 1IIS and 1IIX (Radaev *et al.*, 2001, *J Biol Chem* 276:16469-16477). The extracellular regions of Fc□RIIIb and FcγRIIIa are 96% identical, and therefore the use of the Fc/FcγRIIIb structure is essentially equivalent to use of FcγRIIIa. Nonetheless, for some calculations, a more precise Fc/Fc□RIIIa template structure was constructed by modeling a D129G mutation in the 1IIS and 1E4K structures (referred to as D129G 1IIS and D129G 1E4K template structures). In addition, the structures for human Fc bound to the extracellular domains of human FcγRIIb, human F158 Fc□RIIIa, and mouse FcγRIII were modeled using standard methods, the available Fc□R sequence information, the aforementioned Fc/FcγR structures, as well as structural information for unbound complexes (pdb accession code 1H9V)(Sondermann *et al.*, 2001, *J Mol Biol* 309:737-749) (pdb accession code 1FCG)(Maxwell *et al.*, 1999, *Nat Struct Biol* 6:437-442), Fc□RIIb (pdb accession code 2FCB)(Sondermann *et al.*, 1999, *Embo J* 18:1095-1103), and Fc□RIIIb (pdb accession code 1E4J)(Sondermann *et al.*, 2000, *Nature* 406:267-273.).

[187] Variable positions and amino acids to be considered at those positions were chosen by visual inspection of the aforementioned Fc/FcγR and FcγR structures, and using solvent accessibility information and sequence information. Sequence information of Fcs and FcγRs was particularly useful for determining variable positions at which substitutions may provide distinguishing affinities between activating and inhibitory receptors. Virtually all Cγ2 positions were screened computationally. The Fc structure is a homodimer of two heavy chains (labeled chains A and B in the 1IIS, 1IIX, and 1E4K structures) that each include the hinge and C□2-C□3 domains (shown in Figure 2). Because the Fc□R (labeled chain C in the 1IIS, 1IIX, and 1E4K structures) binds asymmetrically to the Fc homodimer, each chain was often considered separately in design calculations. For some calculations, Fc and/or Fc□R residues proximal to variable position residues were floated, that is the amino acid conformation but not the amino acid identity was allowed to vary in a protein design calculation to allow for conformational adjustments. These are indicated below the table for each set of calculations when relevant. Considered amino acids typically belonged to either the Core, Core XM, Surface, Boundary, Boundary XM, or All 20 classifications, unless noted otherwise. These classifications are defined as follows: Core = alanine, valine, isoleucine, leucine, phenylalanine, tyrosine, tryptophan, and methionine; Core XM = alanine, valine, isoleucine, leucine, phenylalanine, tyrosine, and tryptophan; Surface = alanine, serine, threonine, aspartic acid, asparagine, glutamine, glutamic acid, arginine, lysine and histidine; Boundary = alanine, serine, threonine, aspartic acid, asparagine, glutamine, glutamic acid, arginine, lysine, histidine, valine, isoleucine, leucine, phenylalanine, tyrosine, tryptophan, and methionine; Boundary XM = Boundary = alanine, serine, threonine, aspartic acid, asparagine, glutamine, glutamic acid, arginine, lysine, histidine, valine, isoleucine, leucine, phenylalanine, tyrosine, and tryptophan; All 20 = all 20 naturally occurring amino acids.

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[188] The majority of calculations followed one of two general types of computational screening methods. In one method, the conformations of amino acids at variable positions were represented as a set of backbone-independent side chain rotamers derived from the rotamer library of Dunbrack & Cohen (Dunbrack *et al.*, 1997, *Protein Sci* 6:1661-1681). The energies of all possible combinations of the considered amino acids at the chosen variable positions were calculated using a force field containing terms describing van der Waals, solvation, electrostatic, and hydrogen bond interactions, and the optimal (ground state) sequence was determined using a Dead End Elimination (DEE) algorithm. As will be appreciated by those in the art, the predicted lowest energy sequence is not necessarily the true lowest energy sequence because of errors primarily in the scoring function, coupled with the fact that subtle conformational differences in proteins can result in dramatic differences in stability. However, the predicted ground state sequence is likely to be close to the true ground state, and thus additional favorable diversity can be explored by evaluating the energy of sequences that are close in sequence space and energy around the predicted ground state. To accomplish this, as well as to generate a diversity of sequences for a library, a Monte Carlo (MC) algorithm was used to evaluate the energies of 1000 similar sequences around the predicted ground state. The number of sequences out of the 1000 sequence set that contain that amino acid at that variable position is referred to as the occupancy for that substitution, and this value may reflect how favorable that substitution is. This computational screening method is substantially similar to Protein Design Automation® (PDA®) technology, as described in US 6,188,965; US 6,269,312; US 6,403,312; USSN 09/782,004; USSN 09/927,790; USSN 10/218,102; PCT WO 98/07254; PCT WO 01/40091; and PCT WO 02/25588, and for ease of description, is referred to as PDA® technology throughout the examples. Tables that present the results of these calculations provide for each variable position on the designated chain (column 1) the amino acids considered at each variable position (column 2), the WT Fc amino acid identity at each variable position (column 3), the amino acid identity at each variable position in the DEE ground state sequence (column 4), and the set of amino acids and corresponding occupancy that are observed in the Monte Carlo output (column 5).

[189] Other calculations utilized a genetic algorithm (GA) to screen for low energy sequences, with energies being calculated during each round of "evolution" for those sequences being sampled. The conformations of amino acids at variable and floated positions were represented as a set of side chain rotamers derived from a backbone-independent rotamer library using a flexible rotamer model (Mendes *et al.*, 1999, *Proteins* 37:530-543). Energies were calculated using a force field containing terms describing van der Waals, solvation, electrostatic, and hydrogen bond interactions. This calculation generated a list of 300 sequences which are predicted to be low in energy. To facilitate analysis of the results and library generation, the 300 output sequences were clustered computationally into 10 groups of similar sequences using a nearest neighbor single linkage hierarchical clustering algorithm to assign sequences to related groups based on similarity scores (Diamond, 1995, *Acta Cryst* D51:127-135). That is, all sequences within a group are most similar to

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all other sequences within the same group and less similar to sequences in other groups. The lowest energy sequence from each of these ten clusters are used as a representative of each group, and are presented as results. This computational screening method is substantially similar to Sequence Prediction Algorithm™ (SPA™) technology, as described in (Raha *et al.*, 2000, *Protein Sci* 9:1106-1119); USSN 09/877,695; and USSN 10/071,859, and for ease of description, is referred to as SPA™ technology throughout the examples.

[190] Computational screening was applied to design energetically favorable interactions at the Fc/Fc□R interface at groups of variable positions that mediate or potentially mediate binding with Fc□R. Because the binding interface involves a large number of Fc residues on the two different chains, and because Fc□Rs bind asymmetrically to Fc, residues were grouped in different sets of interacting variable positions, and designed in separate sets of calculations. In many cases these sets were chosen as groups of residues that were deemed to be coupled, that is the energy of one or more residues is dependent on the identity of one or more other residues. Various template structures were used, and in many cases calculations explored substitutions on both chains. For many of the variable position sets, calculations were carried out using both the PDA® and SPA™ technology computational screening methods described. The results of these calculations and relevant parameters and information are presented in Tables 1 – 30 below.

[191] Tables that present the results of these calculations provide for each variable position on the designated chain (column 1) the amino acids considered at each variable position (column 2), the WT Fc amino acid identity at each variable position (column 3), and the amino acid identity at the variable positions for the lowest energy sequence from each cluster group (columns 4-13). Tables 1-59 are broken down into two sets, as labeled below, PDA® and SPA™ technology. Column 4 of the PDA® tables show the frequency of each residue that occurs in the top 1000 sequences during that PDA® run. Thus, in the first row of Table 1, at position 328, when run using boundary amino acids as the set of variable residues for that position, L occurred 330 times in the top 1000 sequence, M occurred 302 times, etc.

[192] In addition, included within the compositions of the invention are antibodies that have any of the listed amino acid residues in the listed positions, either alone or in any combination (note preferred combinations are listed in the claims, the summary and the figures). One preferred combination is the listed amino acids residues in the listed positions in a ground state (sometimes referred to herein as the "global solution", as distinguished from the wild-type). Similarly, residue positions and particular amino acids at those residue positions may be combined between tables.

[193] For SPA™ technology tables, such as Table 4, column 4 is a SPA™ run that results in a protein with the six listed amino acids at the six listed positions (e.g. column 4 is a single protein with

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a wild-type sequence except for 239E, 265G, 267S, 269Y, 270T and 299S. Thus, each of these individual proteins are included within the invention. In addition, combinations between SPA™ proteins, both within tables and between tables, are also included.

[194] In addition, each table shows the presence or absence of carbohydrate, but specifically included are the reverse sequences; e.g. Table 1 is listed for an aglycosylated variant, but these same amino acid changes can be done on a glycosylated variant.

[195] Furthermore, each table lists the template structure used, as well as "floated" residues; for example, Table 2 used a PDA® run that floated C120, C132 and C134.

Table 1

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
328 A	Boundary	L	L	L:330 M:302 E:111 K:62 A:45 Q:39 D:36 S:30 T:28 N:10 R:7
332 A	Surface	I	R	R:247 K:209 Q:130 H:95 E:92 T:59 D:51 N:51 S:42 A:24
328 B	Boundary	L	L	L:321 M:237 T:166 K:73 R:72 S:55 Q:20 D:17 E:13 A:12 V:10 N:4
332 B	Surface	I	E	E:269 Q:180 R:145 K:111 D:97 T:78 N:65 S:28 A:14 H:13

PDA® technology, 1IIS template structure; - carbohydrate

Table 2

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 A	Surface	S	K	E:349 D:203 K:196 A:95 Q:83 S:63 N:10 R:1
265 A	Boundary XM	D	D	D:616 N:113 L:110 E:104 S:25 A:23 Q:9
299 A	Boundary XM	T	I	I:669 H:196 V:135
327 A	Boundary XM	A	S	A:518 S:389 N:67 D:26
265 B	Boundary XM	D	Q	Q:314 R:247 N:118 I:115 A:63 E:55 D:34 S:22 K:21 V:11

PDA® technology; 1IIS template structure; + carbohydrate; floated 120 C, 132 C, 134 C

Table 3

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 A	Surface	S	E	E:872 Q:69 D:39 K:16 A:4
265 A	Boundary XM	D	Y	Y:693 H:111 E:69 D:62 F:29 K:19 R:14 W:2 Q:1
267 A	Boundary XM	S	S	S:991 A:9
269 A	Core XM	E	F	F:938 E:59 Y:3
270 A	Surface	D	E	E:267 T:218 K:186 D:89 Q:88 R:46 S:34 N:29 H:23 A:20
299 A	Boundary XM	T	H	H:486 T:245 K:130 E:40 S:39 D:27 Q:27 A:4 N:2

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PDA® technology; 1IIS template structure; - carbohydrate; floated 120 C, 122 C, 132 C, 133 C, 134 C

Table 4

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 A	Surface	S	E	Q	Q	Q	E	E	E	Q	E	E
265 A	All 20	D	G	G	G	G	G	G	G	G	G	G
267 A	All 20	S	S	S	S	S	S	S	S	S	S	S
269 A	Core	E	Y	Y	A	A	V	Y	A	A	A	A
270 A	Surface	D	T	S	A	S	T	T	T	A	A	A
299 A	All 20	T	S	S	S	S	S	S	S	S	S	S

SPA™ technology; 1IIS template structure; + carbohydrate; floated 120 C, 122 C, 132 C, 133 C, 134 C

Table 5

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
235 A	Boundary XM	L	T	T:195 V:131 L:112 W:107 K:85 F:66 Y:56 E:52 Q:38 S:37 I:34 R:29 H:26 N:23 D:9
296 A	Surface	Y	N	N:322 D:181 R:172 K:76 Y:70 Q:59 E:48 S:40 H:20 T:11 A:1
298 A	Surface	S	T	T:370 R:343 K:193 A:55 S:39
235 B	Boundary XM	L	L	L:922 I:78

PDA® technology; 1IIS template structure; - carbohydrate; floated 119 C, 128 C, 157 C

Table 6

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
235 A	All 20	L	S	S	P	S	S	S	S	S	S	S
296 A	Surface	Y	Q	Q	Q	E	E	Q	E	Q	Q	N
298 A	Surface	S	S	K	K	K	K	S	S	S	K	S
235 B	All 20	L	K	K	K	L	L	L	L	L	L	K

SPA™ technology; 1IIS template structure; + carbohydrate; floated 119 C, 128 C, 157 C

Table 7

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 B	Surface	S	E	K:402 E:282 H:116 T:67 R:47 Q:39 D:26 A:11 S:7 N:3
265 B	Boundary XM	D	W	Y:341 W:283 I:236 V:77 F:36 H:9 T:7 E:4 K:4 A:2 D:1
327 B	Boundary XM	A	R	R:838 K: 86 H:35 E:12 T:10 Q:7 A:6 D:3 N:3
328 B	Core XM	L	L	L:1000
329 B	Core XM	P	P	P:801 A:199
330 B	Core XM	A	Y	Y:918 F:42 L:22 A:18
332 B	Surface	I	I	I:792 E:202 Q:5 K:1

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PDA® technology; 1IIS template structure; - carbohydrate; floated 88 C, 90 C, 113 C, 114 C, 116 C, 160 C, 161 C

Table 8

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 B	Surface	S	D	T	E	E	E	E	E	E	E	E
265 B	All 20	D	G	G	K	G	K	G	G	K	K	G
327 B	All 20	A	K	M	L	L	N	L	K	L	L	L
328 B	Core	L	M	M	M	L	A	M	L	M	L	L
329 B	Core	P	P	P	P	P	P	P	P	P	P	P
330 B	Core	A	L	A	A	A	A	A	A	A	A	A
332 B	Surface	I	I	Q	I	I	Q	Q	E	D	I	I

SPA™ technology; 1IIS template structure; + carbohydrate; floated 88 C, 90 C, 113 C, 114 C, 116 C, 160 C, 161 C

Table 9

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 A	Surface	S	Q	Q	Q	E	Q	E	Q	E	Q	Q
265 A	All 20	D	G	G	G	G	G	G	G	G	G	G
299 A	All 20	T	S	S	A	S	S	S	S	S	S	S
327 A	All 20	A	A	S	S	S	S	S	S	S	A	S
265 B	All 20	D	N	G	G	G	G	G	G	G	G	G

SPA™ technology; 1IIS template structure; - carbohydrate; floated 120 C, 132 C, 134 C

Table 10

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
234 A	Boundary XM	L	K	Y:401 L:260 F:151 I:82 K:63 H:17 Q:11 W:7 R:3 T:2 E:2 V:1
235 A	Boundary XM	L	L	W:777 L:200 K:12 Y:5 I:3 F:2 V:1
234 B	Boundary XM	L	W	W:427 Y:203 L:143 F:74 I:59 E:32 K:23 V:14 D:10 T:7 H:4 R:4
235 B	Boundary XM	L	W	W:380 Y:380 F:135 K:38 L:26 E:15 Q:12 H:8 R:4 T:2

PDA® technology; D129G 1E4K template structure; - carbohydrate; floated 113 C, 116 C, 132 C, 155 C, 157 C

Table 11

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
234 A	All 20	L	G	G	G	G	G	G	G	G	G	G
235 A	All 20	L	T	L	L	L	L	L	L	L	T	L
234 B	All 20	L	G	G	G	G	G	G	G	G	G	G
235 B	All 20	L	S	A	S	A	A	S	S	S	A	A

SPA™ technology; D129G 1E4K template structure; + carbohydrate; floated 113 C, 116 C, 132 C, 155 C, 157 C

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Table 12

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 A	Boundary XM	S	E	E:235 S:122 D:94 Q:93 A:74 K:70 L:67 T:63 N:57 R:51 I:29 V:18 W:15 H:12
328 A	Boundary XM	L	L	L:688 E:121 K:43 Q:41 A:33 D:26 S:14 T:14 N:12 R:8
332 A	Boundary XM	I	W	I:155 W:95 L:82 K:79 E:74 Q:69 H:67 V:63 R:57 T:57 D:45 S:43 N:42 A:35 F:19 Y:18

PDA® technology; D129G 1IIS template structure; - carbohydrate; floated 120 C

Table 13

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 A	All 20	S	L	E	E	Q	E	E	K	K	K	K
328 A	All 20	L	L	Q	L	Q	K	L	L	Q	K	L
332 A	All 20	I	K	K	L	Q	A	K	L	Q	A	Q

SPA™ technology; D129G 1IIS template structure; + carbohydrate; floated 120 C

Table 14

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 B	Boundary XM	S	I	R:195 I:169 L:126 V:91 K:89 E:61 H:52 T:50 Q:42 N:35 S:34 D:30 A:26
328 B	Boundary XM	L	L	L:671 T:165 K:40 S:38 E:28 R:17 Q:17 V:11 A:8 D:5
332 B	Boundary XM	I	I	I:387 E:157 L:151 V:78 Q:63 K:50 R:33 T:29 D:25 A:12 N:8 S:6 W:1

PDA® technology; D129G 1IIS template structure; - carbohydrate; floated 90 C, 160 C, 161 C

Table 15

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 B	All 20	S	T	L	L	L	L	L	L	L	L	L
328 B	All 20	L	M	R	M	D	T	M	L	Q	D	L
332 B	All 20	I	I	D	Q	Q	Q	L	L	T	Q	L

SPA™ technology; D129G 1IIS template structure; + carbohydrate; floated 90 C, 160 C, 161 C

Table 16

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 B	Boundary XM	S	T	T:164 S:159 L:156 E:86 W:76 K:71 D:65 A:52 R:43 H:38 Q:38 N:31 I:14 V:7
328 B	Boundary XM	L	L	L:556 E:114 T:84 K:80 S:69 Q:36 A:31 D:15 R:11 N:4
332 B	Boundary XM	I	W	I:188 W:177 E:97 L:94 T:59 Q:57 V:54 K:52 F:51 D:34 H:33 S:27 R:26 N:18 A:17 Y:16

PDA® technology; D129G 1E4K template structure; - carbohydrate; floated 117 C

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Table 17

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 B	All 20	S	P	S	P	E	L	L	L	L	L	L
328 B	All 20	L	K	K	K	K	K	L	K	K	K	L
332 B	All 20	I	S	S	E	L	L	L	E	L	L	L

SPA™ technology; D129G 1E4K template structure; + carbohydrate; floated 117 C

Table 18

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 A	Boundary XM	S	L	K:196 L:171 I:146 E:88 V:76 R:75 T:50 H:45 D:43 Q:39 S:30 N:22 A:19
328 A	Boundary XM	L	W	L:517 F:230 W:164 H:40 K:29 E:11 R:5 T:4
332 A	Boundary XM	I	E	I:283 L:217 E:178 Q:81 V:64 D:47 T:35 K:27 W:18 R:12 A:10 Y:7 N:7 F:6 S:5 H:3

PDA® technology; D129G 1E4K template structure; - carbohydrate; floated 87 C, 157 C, 158 C

Table 19

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 A	All 20	S	F	Q	E	T	P	P	T	P	P	P
328 A	All 20	L	K	R	R	K	K	M	R	K	M	R
332 A	All 20	I	L	L	I	I	E	I	E	E	I	I

SPA™ technology; D129G 1E4K template structure; + carbohydrate atoms; floated 87 C, 157 C, 158 C

Table 20

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
240 A	Core + Thr	V	V	V:698 M:162 T:140
263 A	Core + Thr	V	V	V:966 T:34
266 A	Core + Thr	V	V	V:983 T:17
325 A	Boundary	N	N	N:943 T:40 A:17
328 A	Boundary	L	L	L:610 M:363 K:27
332 A	Glu	I	E	E:1000

PDA® technology; D129G 1IIS template structure; - carbohydrate; floated 273 A, 275 A, 302 A, 323 A, 134 C

Table 21

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
240 A	All 20	V	V	A	V	V	V	V	V	V	V	V
263 A	All 20	V	V	V	V	V	V	V	V	V	V	V
266 A	All 20	V	I	V	I	I	T	V	V	V	V	I
325 A	All 20	N	A	N	N	N	Q	T	T	Q	N	T

328 A	All 20	L	K	K	L	K	L	K	L	L	L	L
332 A	Glu	I	D	D	D	D	D	D	D	D	D	D

SPA™ technology; D129G 1IIS template structure; + carbohydrate; floated 273 A, 275 A, 302 A, 323 A, 134 C

Table 22

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
240 B	Core + Thr	V	V	V:713 T:287
263 B	Core + Thr	V	V	V:992 T:8
266 B	Core + Thr	V	V	V:976 T:24
325 B	Boundary	N	N	N:453 T:296 A:116 D:96 S:30 V:9
328 B	Boundary	L	L	L:623 M:194 T:100 R:72 K:11
332 B	Glu	I	E	E:1000

PDA® technology; D129G 1IIS template structure; - carbohydrate; floated 273 B, 275 B, 302 B, 323 B, 161 C

Table 23

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
240 B	All 20	V	A	T	A	T	T	A	A	T	T	T
263 B	All 20	V	V	A	A	T	T	V	V	T	A	T
266 B	All 20	V	V	V	V	V	V	V	V	V	I	V
325 B	All 20	N	N	K	K	N	K	K	N	N	N	N
328 B	All 20	L	R	L	L	L	L	L	L	L	L	L
332 A	Glu	I	D	D	D	D	D	D	D	D	D	D

SPA™ technology; D129G 1IIS template structure; + carbohydrate; floated 273 B, 275 B, 302 B, 323 B, 161 C

Table 24

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
240 B	Core + Thr	V	M	V:715 M:271 T:12 I:2
263 B	Core + Thr	V	V	V:992 T:8
266 B	Core + Thr	V	V	V:996 T:4
325 B	Boundary	N	N	N:651 T:232 D:64 A:53
328 B	Boundary	L	M	M:556 L:407 K:37
332 B	Glu	I	E	E:1000

PDA® technology; D129G 1E4K template structure; - carbohydrate; floated 273 B, 275 B, 302 B, 323 B, 131 C

Table 25

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
240 B	All 20	V	T	A	T	A	A	A	A	T	A	A
263 B	All 20	V	T	W	T	T	A	T	T	T	L	L
266 B	All 20	V	L	A	T	T	V	L	T	T	L	V
325 B	All 20	N	A	N	A	A	N	A	A	A	A	A
328 B	All 20	L	L	K	L	L	L	L	L	L	L	L

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332 A	Glu	I	D	D	D	D	D	D	D	D	D	D

SPA™ technology; D129G 1E4K template structure; + carbohydrate; floated 273 B, 275 B, 302 B, 323 B, 131 C

Table 26

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
240 A	Core + Thr	V	V	V:876 T:109 M:15
263 A	Core + Thr	V	V	V:913 T:87
266 A	Core + Thr	V	V	V:969 T:31
325 A	Boundary	N	V	V:491 N:236 T:187 A:35 D:32 S:19
328 A	Boundary	L	L	L:321 W:290 M:271 F:49 K:46 R:23
332 A	Glu	I	E	E:1000

PDA® technology; D129G 1E4K template structure; - carbohydrate; floated 273 A, 275 A, 302 A, 323 A, 158 C

Table 27

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
240 A	All 20	V	A	T	A	A	T	T	A	A	A	T
263 A	All 20	V	T	T	V	V	T	V	L	L	V	T
266 A	All 20	V	V	V	V	V	V	V	V	V	V	V
325 A	All 20	N	Q	N	Q	Q	Q	Q	Q	Q	N	N
328 A	All 20	L	K	M	K	K	K	K	K	K	K	K
332 A	Glu	I	D	D	D	D	D	D	D	D	D	D

SPA™ technology; D129G 1E4K template structure; + carbohydrate; floated 273 A, 275 A, 302 A, 323 A, 158 C

[196] Computational screening calculations were aimed at designing Fc variants to optimize the conformation of the N297 carbohydrate and the Cγ2 domain. By exploring energetically favorable substitutions at positions that interact with carbohydrate, variants can be engineered that sample new, potentially favorable carbohydrate conformations. Fc residues F241, F243, V262, and V264 mediate the Fc/carbohydrate interaction and thus are target positions. The results of these design calculations are presented in Table 28.

Table 28

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
241 A	Core	F	Y	Y:172 M:162 L:144 F:140 W:110 I:97 A:91 V:84
243 A	Core	F	Y	Y:211 L:204 W:199 F:160 M:141 A:85
262 A	Core	V	M	M:302 I:253 V:243 A:202
264 A	Core	V	F	I:159 M:152 V:142 L:140 W:136 F:120 Y:104 A:47

PDA® technology, 1IIS template structure; - carbohydrate

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[197] Computational screening calculations were aimed at designing Fc variants to optimize the angle between the Cγ3 and Cγ2 domains. Residues P244, P245, P247, and W313, which reside at the Cγ2/Cγ3 interface, appear to play a key role in determining the Cγ2-Cγ3 angle and the flexibility of the domains relative to one another. By exploring energetically favorable substitutions at these positions, variants can be designed that sample new, potentially favorable angles and levels of flexibility. The results of these design calculations are presented in Table 29.

Table 29

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
244 A	Boundary	P	H	K:164 H:152 R:110 M:100 S:92 N:57 A:54 D:50 Q:49 T:46 E:37 V:30 L:27 W:23 F:9
245 A	Boundary	P	A	A:491 S:378 N:131
247 A	Boundary	P	V	V:156 T:125 K:101 E:87 Q:79 R:78 S:76 A:72 D:72 H:60 M:47 N:47
313 A	Boundary	W	W	W:359 F:255 Y:128 M:114 H:48 K:29 T:24 A:11 E:10 V:10 S:9 Q:3

PDA® technology; 1IIS template structure; - carbohydrate

[198] In addition to the above calculations using PDA® and SPA™ computational screening methods, additional calculations using solely an electrostatic potential were used to computationally screen Fc variants. Calculations with Coulomb's law and Debye-Huckel scaling highlighted a number of positions in the Fc for which amino acid substitutions would favorably affect binding to one or more FcγRs, including positions for which replacement of a neutral amino acid with a negatively charged amino acid may enhance binding to FcγRIIIa, and for which replacement of a positively charged amino acid with a neutral or negatively charged amino acid may enhance binding to FcγRIIIa. These results are presented in Table 30.

Table 30

Replacement of a + residue with a - residue	Replacement of a neutral residue with a - residue
H268	S239
K326	Y296
K334	A327
	I332

Coulomb's law and Debye-Huckel scaling; 1IIS template structure; + carbohydrate

[199] Computational screening calculations were carried out to optimize aglycosylated Fc, that is to optimize Fc structure, stability, solubility, and Fc/FcγR affinity in the absence of the N297 carbohydrate. Design calculations were aimed at designing favorable substitutions in the context of

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the aglycosylated Fc template structure at residue 297, residues proximal to it, residues at the Fc/FcγR interface, and residues at the Fc/carbohydrate interface. Variable positions were grouped in different sets of interacting variable positions and designed in separate sets of calculations, and various template structures were used. For many of the variable position sets, calculations were carried out using both the PDA® and SPA™ computational screening methods. The results of these calculations and relevant information are presented in Tables 31 - 53 below.

Table 31

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
265 A	Boundary XM	D	Y	Y:531 F:226 W:105 H:92 K:21 D:16 E:6 T:3
297 A	Boundary XM	N	D	A:235 S:229 D:166 E:114 N:92 Y:57 F:55 Q:25 H:10 T:7 K:6 L:3 R:1
299 A	Boundary XM	T	L	L:482 Y:186 F:131 T:55 S:51 K:31 H:22 A:18 E:14 Q:10
297 B	Boundary XM	N	I	I:299 K:147 V:85 R:82 W:71 N:65 D:35 E:35 Q:34 S:32 L:31 H:30 T:28 A:26

PDA® technology; 1IIS template structure; - carbohydrate; floated 122 C, 129 C, 132 C, 155 C

Table 32

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
265 A	All 20	D	G	G	G	G	G	G	G	G	G	G
297 A	All 20	N	A	T	A	E	K	K	A	A	N	N
299 A	All 20	T	S	K	S	K	F	F	F	F	F	S
297 B	All 20	N	K	K	K	K	K	K	K	K	K	K

SPA™ technology; 1IIS template structure; - carbohydrate; floated 122 C, 129 C, 132 C, 155 C

Table 33

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 A	Surface	S	E	E:928 Q:65 D:7
265 A	Boundary XM	D	W	W:709 Y:248 F:43
296 A	Surface	Y	H	H:449 Y:146 E:137 D:89 K:64 N:32 T:30 R:25 Q:23 S:5
297 A	Surface	N	E	E:471 H:189 D:102 T:97 K:96 R:22 Q:15 S:8
298 A	Boundary XM	S	R	R:353 T:275 K:269 A:56 S:38 E:5 Q:2 H:2
299 A	Boundary XM	T	F	Y:398 F:366 L:217 H:15 K:4

PDA® technology; D129G 1IIS template structure; - carbohydrate; floated 120 C, 122 C, 128 C, 132 C, 155 C

Table 34

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 A	All 20	S	E	Q	Q	E	Q	Q	Q	Q	Q	Q
265 A	All 20	D	G	G	G	G	G	G	G	G	G	G
296 A	All 20	Y	D	Q	N	N	Q	N	N	N	Q	N

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297 A	All 20	N	A	A	N	A	D	D	E	N	N	E
298 A	All 20	S	K	K	K	S	K	K	K	K	S	K
299 A	All 20	T	S	Y	F	S	Y	F	K	F	S	K

SPA™ technology; D129G 1IIS template structure; - carbohydrate; floated 120 C, 122 C, 128 C, 132 C, 155 C

Table 35

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 B	Surface	S	E	E:417 T:122 D:117 Q:94 R:84 S:63 K:47 H:29 N:19 A:8
265 B	Boundary XM	D	W	W:865 Y:79 F:55 K:1
296 B	Surface	Y	Y	Y:549 H:97 D:80 S:75 N:48 E:45 K:32 R:30 Q:28 A:16
297 B	Surface	N	R	R:265 H:224 E:157 K:154 Q:75 D:47 T:34 N:24 S:13 A:7
298 B	Boundary XM	S	V	V:966 D:10 T:8 A:8 N:4 S:4
299 B	Boundary XM	T	Y	Y:667 F:330 H:3

PDA® technology; D129G 1E4K template structure; - carbohydrate; floated 117 C, 119 C, 125 C, 129 C, 152 C

Table 36

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 B	All 20	S	S	R	E	K	S	S	E	E	E	K
265 B	All 20	D	A	D	K	Y	A	A	F	F	K	Y
296 B	All 20	Y	A	A	A	A	A	A	A	A	A	A
297 B	All 20	N	T	S	T	T	E	E	E	S	E	E
298 B	All 20	S	G	G	G	G	G	G	G	G	G	G
299 B	All 20	T	L	F	E	E	Y	F	Y	F	Y	Y

SPA™ technology; D129G 1E4K template structure; - carbohydrate; floated 117 C, 119 C, 125 C, 129 C, 152 C

Table 37

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 A	Surface	S	E	E:868 Q:92 D:38 K:1 N:1
265 A	Boundary XM	D	W	W:575 Y:343 F:66 H:15 K:1
296 A	Surface	Y	H	H:489 Y:103 R:98 K:97 Q:64 D:63 T:41 N:38 E:7
297 A	Asp	N	D	D:1000
298 A	Boundary XM	S	R	R:340 K:262 T:255 A:59 S:57 E:11 Q:10 H:6
299 A	Boundary XM	T	F	Y:375 F:323 L:260 H:24 K:18

PDA® technology; D129G 1IIS template structure; - carbohydrate; floated 120 C, 122 C, 128 C, 132 C, 155 C

Table 38

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 A	All 20	S	E	Q	E	E	E	E	E	E	Q	E

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265 A	All 20	D	G	G	G	G	G	G	G	G	G	G
296 A	All 20	Y	E	N	Q	E	N	Q	Q	Q	Q	N
297 A	Asp	N	D	D	D	D	D	D	D	D	D	D
298 A	All 20	S	K	S	K	S	K	K	K	S	K	K
299 A	All 20	T	S	K	Y	S	F	F	F	F	F	K

SPA™ technology; D129G 1IIS template structure; - carbohydrate; floated 120 C, 122 C, 128 C, 132 C, 155 C

Table 39

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 B	Surface	S	E	E:318 Q:123 T:109 D:108 R:93 S:89 K:69 N:40 H:38 A:13
265 B	Boundary XM	D	W	W:745 Y:158 F:85 K:9 E:1 R:1 H:1
296 B	Surface	Y	Y	Y:390 H:127 S:83 R:81 K:78 N:65 D:55 E:49 Q:44 A:26 T:2
297 B	Asp	N	D	D:1000
298 B	Boundary XM	S	V	V:890 T:35 A:29 D:19 S:16 N:10 E:1
299 B	Boundary XM	T	Y	Y:627 F:363 H:10

PDA® technology; D129G 1E4K template structure; - carbohydrate; floated 117 C, 119 C, 125 C, 129 C, 152 C

Table 40

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 B	All 20	S	K	E	E	Q	E	K	Q	E	K	Q
265 B	All 20	D	F	K	K	A	K	Y	W	K	L	F
296 B	All 20	Y	A	A	A	A	A	A	A	A	A	A
297 B	Asp	N	D	D	D	D	D	D	D	D	D	D
298 B	All 20	S	G	G	G	G	G	G	G	G	G	G
299 B	All 20	T	Y	Y	Y	Y	Y	Y	F	F	Y	Y

SPA™ technology; D129G 1E4K template structure; - carbohydrate; floated 117 C, 119 C, 125 C, 129 C, 152 C

Table 41

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 A	Boundary XM	S	E	E:312 L:148 D:102 Q:98 K:64 I:61 S:57 A:44 T:39 N:29 R:23 V:18 W:5
265 A	Boundary XM	D	W	W:363 Y:352 F:139 H:77 K:39 R:14 D:11 E:4 Q:1
297 A	Asp	N	D	D:1000
299 A	Boundary XM	T	Y	Y:309 F:224 L:212 H:96 K:92 E:28 Q:20 R:16 T:2 S:1

PDA® technology; D129G 1IIS template structure; - carbohydrate; floated 120 C, 122 C, 132 C, 155 C

Table 42

Position	Considered	WT	1	2	3	4	5	6	7	8	9	10

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	Amino Acids											
239 A	All 20	S	E	L	L	L	E	E	E	Q	L	E
265 A	All 20	D	G	G	G	G	G	G	G	G	G	G
297 B	Asp	N	D	D	D	D	D	D	D	D	D	D
299 A	All 20	T	S	K	K	F	F	F	K	F	K	F

SPA™ technology; D129G 1IIS template structure; - carbohydrate; floated 120 C, 122 C, 132 C, 155 C

Table 43

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 B	Boundary XM	S	L	L:194 T:122 S:120 E:111 D:79 K:71 A:62 Q:57 R:43 H:43 N:37 I:24 W:24 V:13
265 B	Boundary XM	D	W	Y:248 W:233 F:198 K:84 D:57 E:55 H:42 R:28 Q:20 A:10 T:10 N:8 S:7
297 B	Asp	N	D	D:1000
299 B	Boundary XM	T	Y	Y:493 F:380 H:76 T:31 E:10 D:4 A:3 S:3

PDA® technology; D129G 1E4K template structure; - carbohydrate; floated 117 C, 119 C, 129 C, 152 C

Table 44

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 B	All 20	S	R	E	P	L	L	F	P	P	L	L
265 B	All 20	D	D	K	S	F	S	Y	A	M	A	D
297 B	Asp	N	D	D	D	D	D	D	D	D	D	D
299 B	All 20	T	Y	Y	Y	Y	E	Y	Y	Y	Y	Y

SPA™ technology; D129G 1E4K template structure; - carbohydrate; floated 117 C, 119 C, 129 C, 152 C

Table 45

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 A	Boundary XM	S	E	E:251 L:125 D:120 Q:112 S:73 K:65 I:61 A:58 T:45 N:35 R:28 V:23 W:4
265 A	Boundary XM	D	Y	Y:216 H:153 K:135 D:109 W:104 F:86 R:54 T:38 E:29 Q:22 A:21 N:17 S:13 L:3
297 A	Asp	N	D	D:1000

PDA® technology; D129G 1IIS template structure; - carbohydrate; floated 299 A, 120 C, 122 C, 132 C, 155 C

Table 46

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 A	All 20	S	S	L	E	L	Q	Q	E	Q	Q	E
265 A	All 20	D	G	G	G	G	G	G	G	G	G	G
297 A	Asp	N	D	D	D	D	D	D	D	D	D	D

SPA™ technology; D129G 1IIS template structure; - carbohydrate; floated 299 A, 120 C, 122 C, 132 C, 155 C

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Table 47

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
239 B	Boundary XM	S	L	L:158 S:137 T:125 E:115 D:86 K:75 A:62 Q:56 H:43 R:39 N:35 W:30 I:24 V:15
265 B	Boundary XM	D	Y	Y:188 W:159 F:156 D:122 K:77 E:71 H:61 Q:44 R:39 A:24 S:22 N:19 T:18
297 B	Asp	N	D	D:1000

PDA® technology; D129G 1E4K template structure; - carbohydrate; floated 299 B, 117 C, 119 C, 129 C, 152 C

Table 48

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
239 B	All 20	S	S	E	P	P	E	S	P	L	F	L
265 B	All 20	D	A	K	A	M	K	F	Y	D	F	F
297 B	Asp	N	D	D	D	D	D	D	D	D	D	D

SPA™ technology; D129G 1E4K template structure; - carbohydrate; floated 299 B, 117 C, 119 C, 129 C, 152 C

Table 49

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
297 A	Asp	N	D	D:1000
299 A	Boundary XM	T	Y	T:123 Y:64 H:64 K:64 Q:64 F:64 R:63 D:63 E:63 S:63 L:63 N:62 I:57 A:54 V:52 W:17

PDA® technology; D129G 1IIS template structure; - carbohydrate; floated 239 A, 265 A, 120 C, 122 C, 132 C, 155 C

Table 50

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
297 A	Asp	N	D	D	D	D	D	D	D	D	D	D
299 A	All 20	T	K	K	K	K	F	F	K	K	K	K

SPA™ technology; D129G 1IIS template structure; - carbohydrate; floated 239 A, 265 A, 120 C, 122 C, 132 C, 155 C

Table 51

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
297 B	Asp	N	D	D:1000
299 B	Boundary XM	T	Y	T:123 F:64 Y:64 H:64 S:63 N:61 Q:61 D:61 E:60 K:58 V:57 A:57 R:54 I:52 L:51 W:50

PDA® technology; D129G 1E4K template structure; - carbohydrate; floated 239 B, 265 B, 117 C, 119 C, 129 C, 152 C

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Table 52

Position	Considered Amino Acids	WT	1	2	3	4	5	6	7	8	9	10
297 B	Asp	N	D	D	D	D	D	D	D	D	D	D
299 B	All 20	T	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

SPA™ technology; D129G 1E4K template structure; - carbohydrate; floated 239 B, 265 B, 117 C, 119 C, 129 C, 152 C

[200] Computational screening calculations were carried out to optimize aglycosylated Fc by designing favorable substitutions at residues that are exposed to solvent in the absence of glycosylation such that they are stable, maintain Fc structure, and have no tendency to aggregate. The N297 carbohydrate covers up the exposed hydrophobic patch that would normally be the interface for a protein-protein interaction with another Ig domain, maintaining the stability and structural integrity of Fc and keeping the Cγ2 domains from aggregating across the central axis. Key residues for design are F241, F243, V262, and V264, which reside behind the carbohydrate on Cγ2, in addition to residues such as L328, I332, and I336, which are exposed nonpolar residues facing inward towards the opposed Cγ2 domain, that were considered in previously presented calculations. The importance of these Cγ2 residues is supported by noting that the corresponding residues in the Cγ3 domain by sequence alignment either mediate the nonpolar interaction between the two Cγ3 domains or are buried in the Cγ3 core. The results of these design calculations are presented in Table 53.

Table 53

Position	Considered Amino Acids	WT	Ground State	Sequences Around Ground State
241 A	Surface	F	E	E:190 R:172 K:138 H:117 T:93 Q:91 D:85 S:49 N:49 A:16
243 A	Surface	F	R	R:190 H:164 Q:152 E:149 K:92 T:71 D:64 N:58 S:42 A:18
262 A	Surface	V	D	D:416 E:164 N:138 Q:87 T:83 R:44 S:32 K:24 A:11 H:1
264 A	Surface	V	H	R:368 H:196 K:147 E:108 Q:68 T:34 N:33 D:25 S:15 A:6

PDA® technology; 1IIS template structure; - carbohydrate

[201] In a final set of calculations, a SPA™ computational screening method was applied to evaluate the replacement of all chosen variable positions with all 20 amino acids. The lowest energy rotamer conformation for all 20 amino acids was determined, and this energy was defined as the energy of substitution for that amino acid at that variable position. These calculations thus provided an energy of substitution for each of the 20 amino acids at each variable position. These data were

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useful for a variety of design goals aimed at both glycosylated and aglycosylated Fc, including optimization of Fc/FcγR affinity, Fc stability, Fc solubility, carbohydrate conformation, and hinge conformation. Furthermore, because these calculations provide energies for both favorable and unfavorable substitutions, they guide substitutions that may enable differential binding to activating versus inhibitory FcγRs. Various template structures were used, and calculations explored substitutions on both chains. The results of these calculations and relevant parameters and information are presented in Tables 54 - 59 below. Column 1 lists the variable positions on chain A and B of the 1IIS template structure. Column 2 lists the wild-type amino acid identity at each variable position. The remaining 20 columns provide the energy for each of the natural 20 amino acids (shown in the top row). All substitutions were normalized with respect to the lowest energy substitution, which was set to 0 energy. For example in Table 54, for L235 on chain A, serine is the lowest energy substitution, and L235A is 0.9 kcal/mol less stable than L235S. Extremely high energies were set to 20 kcal/mol for energies between 20 – 50 kcal/mol, and 50 kcal/mold for energies greater than 50 kcal/mol. Favorable substitutions may be considered to be the lowest energy substitution for each position, and substitutions that have small energy differences from the lowest energy substitution, for example substitutions within 1-2, 1-3, 1-5, or 1-10 kcal/mol.

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Table 54

Pos	WT	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
235 A	L	0.9	2.8	2.8	1.5	3.2	3.2	3.4	4.9	1.6	2.1	3.2	0.9	0.3	1.3	0.7	0.0	1.7	4.3	6.5	3.2
236 A	G	0.0	1.9	5.1	6.7	10.0	2.3	4.3	17.2	5.7	20.0	4.6	3.2	12.6	5.6	6.1	0.6	6.2	12.0	6.7	20.0
237 A	G	20.0	20.0	20.0	50.0	50.0	0.0	50.0	50.0	20.0	50.0	20.0	20.0	50.0	50.0	50.0	20.0	20.0	50.0	50.0	50.0
239 A	S	0.2	4.3	2.6	0.0	12.8	4.5	6.9	11.3	1.7	0.1	2.1	1.7	7.9	1.2	2.6	0.3	5.7	11.0	20.0	20.0
265 A	D	9.0	8.1	6.3	7.8	5.1	0.0	7.3	50.0	8.2	9.9	7.7	6.0	50.0	9.0	8.5	7.8	20.0	50.0	20.0	5.8
267 A	S	2.1	3.3	7.3	1.4	50.0	7.3	20.0	20.0	0.9	2.2	5.0	4.8	0.0	2.2	3.1	2.9	20.0	20.0	50.0	50.0
269 A	E	0.5	2.1	1.3	0.6	1.6	3.9	2.0	1.2	1.1	1.3	2.7	0.0	50.0	0.6	1.1	0.3	0.8	1.0	5.6	1.2
270 A	D	0.3	2.8	2.3	2.0	4.0	4.0	3.4	2.4	1.2	0.0	2.3	2.1	20.0	2.0	2.3	1.4	1.8	4.2	5.4	6.0
296 A	Y	2.7	2.0	1.4	0.0	50.0	0.0	50.0	4.6	2.1	2.4	3.3	1.2	50.0	0.2	1.5	1.3	4.6	4.4	16.3	18.2
298 A	S	0.7	2.4	6.7	3.4	20.0	3.9	20.0	6.7	0.0	4.1	1.4	4.1	50.0	1.8	1.1	0.2	2.2	6.3	17.8	20.0
299 A	T	0.6	2.8	11.5	10.1	20.0	6.1	20.0	10.7	7.1	20.0	4.3	6.8	50.0	6.3	12.0	0.0	3.0	7.1	14.8	20.0
234 B	L	2.1	3.2	4.1	4.2	1.6	5.3	0.1	0.7	0.6	1.0	2.0	1.7	50.0	2.8	0.3	2.3	1.7	2.6	13.0	0.0
235 B	L	0.6	2.3	2.5	0.7	5.4	4.8	1.4	3.6	0.1	0.0	2.0	1.7	16.6	0.5	1.2	0.7	0.7	5.3	6.8	5.5
236 B	G	3.1	1.3	4.4	8.2	5.2	0.0	1.9	20.0	3.1	20.0	4.1	2.7	50.0	3.7	16.0	1.2	20.0	20.0	20.0	11.3
237 B	G	20.0	50.0	50.0	50.0	50.0	0.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
239 B	S	0.9	2.4	3.4	1.8	5.4	5.6	2.7	3.0	0.9	0.0	2.0	1.6	50.0	1.8	1.8	1.4	1.4	5.1	20.0	5.3
265 B	D	4.5	5.1	4.6	4.6	4.9	0.0	3.8	9.0	2.0	2.5	4.1	2.1	50.0	4.5	5.1	4.4	5.9	9.2	11.4	5.8
327 B	A	1.8	3.4	4.7	3.9	20.	7.0	20.	20.	0.8	0.0	1.9	1.5	20.	3.0	2.6	3.2	20.	20.	20.	20.

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						0		0	0					0				0	0	0	0
328 B	L	3.7	3.6	4.0	3.7	50.0	8.4	6.8	50.0	3.8	0.0	2.1	4.1	50.0	3.6	8.1	4.9	3.0	12.5	50.0	50.0
329 B	P	3.4	8.6	20.0	20.0	50.0	8.0	16.8	50.0	20.0	20.0	16.9	20.0	0.0	20.0	20.0	1.3	17.1	16.5	50.0	50.0
330 B	A	0.5	2.0	2.6	0.5	2.4	3.8	1.4	4.2	0.0	2.0	2.2	0.8	20.0	0.1	0.6	0.9	0.3	5.1	8.0	2.7
332 B	I	1.5	2.7	1.2	1.6	11.9	6.8	12.9	1.2	2.9	0.0	1.4	1.7	50.0	1.3	4.9	1.8	1.7	3.0	20.0	20.0

SPA™ technology; 1IIS template structure; + carbohydrate atoms, no floated positions

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Table 55

Pos	WT	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
235 A	L	0.9	2.8	2.6	1.7	3.3	3.3	3.4	5.0	1.6	2.1	3.3	1.0	0.3	1.4	1.8	0.0	1.9	3.6	6.6	3.3
236 A	G	0.0	1.7	5.2	6.0	11.3	2.3	4.4	17.2	5.8	19.0	4.9	3.3	8.2	5.6	6.0	0.8	5.6	11.8	6.6	20.0
237 A	G	20.00	20.00	20.00	50.00	50.00	0.0	50.00	50.00	20.00	50.00	20.00	20.00	50.00	50.00	50.00	20.00	20.00	50.00	50.00	50.0
238 A	P	8.6	8.0	10.5	13.4	6.4	0.0	5.0	50.0	12.4	11.3	9.7	9.3	3.2	12.4	20.0	8.6	50.0	50.0	20.0	8.4
239 A	S	0.1	4.2	2.5	0.0	20.0	4.5	9.0	10.8	1.8	0.2	2.1	1.8	9.1	1.3	2.5	0.3	5.7	10.7	20.0	19.7
240 A	V	1.3	2.4	2.3	6.3	20.0	7.2	20.0	5.1	10.8	6.2	5.7	2.0	1.1	9.5	13.1	2.5	0.5	0.0	20.0	20.0
241 A	F	0.1	1.6	1.2	0.3	0.2	4.1	1.2	10.0	1.3	0.1	2.1	0.4	14.7	0.5	1.1	0.1	0.0	8.3	3.6	0.4
242 A	L	3.0	3.4	5.5	8.3	14.4	8.5	11.1	3.3	13.9	2.2	2.7	5.5	0.9	7.9	17.1	3.8	2.3	0.0	20.0	17.5
243 A	F	1.6	2.2	2.7	0.2	1.4	5.6	2.5	0.0	2.2	2.0	3.0	2.3	10.2	0.5	1.6	1.3	0.9	1.2	5.3	1.6
244 A	P	1.2	1.8	3.8	0.8	10.2	3.8	4.6	20.0	0.2	2.9	2.0	2.8	2.0	0.9	1.7	0.0	19.3	20.0	7.6	12.2
245 A	P	3.9	20.00	20.00	20.00	20.00	9.1	20.00	20.00	20.00	20.00	20.00	20.00	0.0	20.00	20.00	8.0	20.00	50.00	20.00	20.00
246 A	K	1.3	2.7	2.0	2.0	2.9	5.7	2.9	1.4	1.4	1.5	3.1	0.2	0.0	1.2	1.5	1.7	1.4	1.2	5.4	3.0
247 A	P	1.2	2.1	0.3	0.7	4.0	3.9	3.7	1.8	1.6	1.7	3.3	0.0	0.5	0.9	1.5	0.7	1.1	1.3	6.9	3.7
248 A	K	0.9	2.7	1.5	0.8	3.1	4.7	3.4	3.3	2.0	1.9	2.6	1.2	3.6	1.5	2.3	0.7	0.0	2.5	5.6	2.7
249 A	D	1.2	3.7	1.6	0.0	20.0	7.3	19.7	50.0	1.7	20.0	2.2	1.4	20.0	1.5	3.4	2.5	18.3	50.0	20.0	20.0
250 A	T	0.0	1.8	3.8	5.8	50.0	6.0	20.0	4.5	6.3	6.3	0.3	3.2	50.0	8.7	9.3	1.8	1.3	1.9	20.0	50.0
251 A	L	1.1	1.9	1.2	0.5	5.8	5.1	1.9	5.6	0.9	0.7	2.4	1.4	50.0	0.0	1.4	0.5	0.8	6.9	8.9	5.8
252	M	0.3	1.2	0.6	0.0	3.0	3.8	3.4	3.9	1.0	0.3	2.2	0.3	17.	0.1	1.1	0.1	0.2	4.6	4.2	3.3

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A																									
253 A	I	0.7	1.7	1.1	0.2	1.8	3.5	2.2	2.0	0.3	1.2	0.8	0.8	0.3	0.0	1.1	0.3	0.5	2.8	2.4	1.9
254 A	S	0.7	1.7	0.4	0.7	2.2	3.6	2.0	0.3	1.2	1.9	2.4	0.0	20.0	0.3	1.2	0.3	0.8	0.7	3.8	1.9
255 A	R	1.4	2.8	2.4	2.5	0.2	5.4	1.1	17.0	1.0	2.2	1.5	1.7	50.0	2.1	0.0	2.3	50.0	17.2	4.0	0.5
256 A	T	0.6	1.8	1.2	1.1	2.7	3.4	2.1	1.4	0.7	1.5	2.4	0.0	0.4	0.1	0.2	0.4	0.9	1.2	5.6	2.7
257 A	P	0.0	7.8	20.0	12.9	50.0	6.2	50.0	20.0	12.3	12.8	14.4	20.0	0.1	13.1	20.0	2.9	16.0	20.0	50.0	50.0
258 A	E	0.0	1.6	4.8	2.6	1.0	4.3	2.2	14.8	4.4	6.2	3.2	2.9	10.4	7.4	6.0	1.0	6.2	17.6	20.0	1.0
259 A	V	3.9	4.3	5.1	8.7	20.0	10.3	6.8	2.3	9.6	2.8	6.2	4.1	50.0	9.2	20.0	5.2	2.1	0.0	20.0	20.0
260 A	T	1.7	2.3	3.3	1.1	20.0	6.6	8.6	0.0	0.2	1.8	2.8	1.8	1.1	0.8	0.9	1.7	0.4	1.9	7.1	20.0
261 A	C	0.0	20.0	20.0	20.0	20.0	3.9	20.0	20.0	20.0	20.0	20.0	20.0	50.0	20.0	20.0	3.6	20.0	20.0	20.0	20.0
262 A	V	1.9	3.2	0.0	3.3	20.0	7.2	20.0	8.3	2.9	2.9	2.2	0.6	50.0	3.8	5.2	3.4	3.0	1.7	20.0	20.0
263 A	V	2.2	2.7	6.0	17.4	20.0	8.8	20.0	10.0	7.1	7.6	16.9	5.2	50.0	19.8	17.7	2.8	1.4	0.0	20.0	20.0
264 A	V	1.9	3.3	2.8	2.2	0.0	6.4	2.1	0.7	2.6	0.9	2.7	2.1	2.3	2.6	2.7	2.2	1.1	0.6	3.9	0.1
265 A	D	9.0	8.1	5.9	8.6	5.3	0.0	7.3	50.0	7.9	9.7	7.5	5.5	50.0	10.2	8.6	7.9	20.0	50.0	20.0	5.7
266 A	V	4.9	5.3	7.1	12.1	20.0	11.2	20.0	0.4	12.2	20.0	8.8	7.1	50.0	12.2	20.0	6.1	3.8	0.0	20.0	20.0
267 A	S	2.3	3.5	7.2	1.3	50.0	7.4	20.0	20.0	0.7	1.4	3.9	4.7	0.0	2.3	3.1	3.0	20.0	20.0	50.0	50.0
268 A	H	1.2	1.9	2.2	1.5	3.7	5.0	4.9	0.4	0.5	3.7	2.7	1.7	0.0	1.4	1.7	1.1	0.2	0.9	6.1	3.7
269 A	E	0.3	1.9	1.3	0.5	1.3	3.7	1.9	1.1	0.8	1.2	2.5	0.0	50.0	0.6	0.8	0.2	0.6	0.7	4.0	1.0
270 A	D	0.2	2.6	2.1	1.9	5.2	3.9	3.1	2.1	1.2	0.0	2.2	1.9	20.0	1.9	1.8	1.2	1.7	4.1	5.1	7.0

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Table 55 (continued)

Pos	WT	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
271 A	P	0.0	5.3	8.1	9.3	20.0	3.1	9.1	20.0	6.0	9.5	5.3	7.3	5.9	5.9	5.9	1.6	4.1	15.2	20.0	20.0
272 A	Q	0.8	1.9	0.9	1.2	3.0	3.2	3.7	3.7	1.6	1.8	3.2	0.3	50.0	1.1	1.6	0.0	1.0	3.5	4.0	3.4
273 A	V	1.2	2.9	1.8	20.0	20.0	7.1	20.0	6.8	20.0	20.0	20.0	0.0	2.8	20.0	20.0	2.1	1.4	1.7	20.0	20.0
274 A	K	0.4	1.8	1.4	0.8	1.9	3.9	2.4	1.4	0.7	1.1	2.9	0.9	20.0	0.0	0.1	0.0	0.4	0.7	3.3	2.3
275 A	F	8.0	9.5	10.3	9.5	0.0	13.5	5.1	10.1	6.2	6.3	6.0	9.1	6.1	9.1	15.1	9.6	7.2	6.1	13.5	4.3
276 A	N	1.3	2.4	2.4	2.2	0.8	5.1	0.8	1.2	0.6	2.3	2.5	1.8	50.0	1.6	2.5	1.2	0.0	0.3	4.2	3.6
277 A	W	5.5	7.4	8.4	6.4	15.4	11.2	3.2	8.2	1.9	3.9	3.6	6.6	3.5	5.5	15.4	6.9	6.1	14.1	0.0	20.0
278 A	Y	1.6	2.7	3.9	1.6	1.0	7.3	3.4	17.7	1.4	7.5	2.1	0.0	50.0	1.9	2.2	2.6	9.9	20.0	15.8	1.4
279 A	V	3.1	4.1	4.0	2.2	20.0	8.1	9.7	8.5	0.0	1.4	3.1	3.3	20.0	1.9	4.6	4.3	3.4	4.2	20.0	20.0
280 A	D	1.8	2.6	2.7	0.2	11.5	2.9	8.8	20.0	3.4	3.2	2.8	3.8	50.0	0.0	3.7	0.6	6.8	12.7	11.9	11.4
281 A	G	50.0	50.0	50.0	50.0	50.0	0.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
282 A	V	0.9	2.1	1.6	1.1	2.9	4.2	3.5	1.4	1.5	1.8	3.6	0.4	18.9	0.5	1.0	0.0	0.6	0.9	4.7	3.1
283 A	E	0.7	1.6	0.7	0.5	1.0	4.4	1.4	0.4	1.2	1.8	1.9	0.0	0.4	0.6	1.5	0.4	0.3	1.2	4.1	0.9
284 A	V	0.0	2.2	3.1	1.2	20.0	5.0	20.0	4.0	0.7	2.6	0.8	2.6	50.0	0.8	0.7	0.8	0.1	1.5	20.0	20.0
285 A	H	0.2	1.4	3.1	1.3	3.0	2.0	2.4	3.6	1.1	2.6	3.0	0.7	2.2	0.2	0.8	0.0	1.1	4.7	4.9	4.0
286 A	N	0.8	2.5	1.2	1.1	2.4	4.7	2.7	2.1	0.0	0.7	1.8	0.6	20.0	1.2	0.7	0.9	1.7	2.1	5.2	2.7
287 A	A	0.6	2.6	5.8	3.3	10.4	5.4	9.1	11.3	0.0	4.4	1.3	3.6	50.0	2.6	2.3	1.0	1.9	12.5	9.1	10.4
288	K	0.8	2.6	2.0	1.3	3.0	3.4	3.8	2.3	1.4	1.7	2.5	0.3	50.	0.5	1.3	0.0	0.4	2.0	4.5	3.6

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A																							
289 A	T	0.3	1.9	4.7	1.1	3.1	3.6	2.9	10.5	0.4	2.7	1.6	2.1	8.2	1.2	2.0	0.0	0.4	12.0	3.9	3.2
290 A	K	1.7	2.2	0.5	0.6	3.0	1.3	3.0	3.7	1.7	2.1	3.2	0.0	50.0	0.7	2.0	0.3	1.3	3.3	5.6	3.3
291 A	P	1.6	3.1	1.8	0.5	1.9	5.5	1.8	0.1	0.5	1.5	1.2	1.2	0.7	0.0	2.9	2.2	0.9	1.3	2.6	0.9
292 A	R	1.1	2.2	3.1	0.8	5.9	4.4	8.0	5.0	0.0	1.6	2.1	1.1	8.4	0.2	0.4	1.0	1.3	4.7	8.3	5.7
293 A	E	2.2	6.5	9.0	17.9	16.3	0.0	13.2	50.0	12.8	10.3	10.3	7.2	5.5	15.1	14.5	3.5	20.0	50.0	14.5	17.1
294 A	E	1.5	2.1	2.1	0.7	8.1	2.8	3.3	2.0	2.6	1.8	2.8	1.0	50.0	1.3	1.3	0.5	0.0	3.4	11.2	10.2
295 A	Q	50.0	50.0	50.0	50.0	50.0	0.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
296 A	Y	2.8	2.3	1.1	0.4	50.0	0.0	50.0	4.6	2.2	2.3	3.1	0.9	50.0	0.2	1.8	1.3	4.7	4.8	18.2	20.0
297 A	N	0.0	6.5	8.4	5.3	20.0	3.4	20.0	13.8	2.7	20.0	4.8	9.3	50.0	4.4	4.4	1.5	1.6	15.5	20.0	20.0
298 A	S	0.8	2.4	5.7	2.2	20.0	3.7	20.0	6.2	0.9	9.2	1.8	3.3	50.0	1.7	2.1	0.0	2.2	7.3	15.6	20.0
299 A	T	1.9	3.4	6.0	3.1	1.0	7.1	2.9	3.1	0.0	2.7	2.6	3.6	50.0	2.2	2.5	1.1	2.2	5.4	3.6	1.4
300 A	Y	2.8	2.9	2.7	4.5	20.0	4.0	7.5	13.1	1.2	0.0	2.2	2.3	50.0	3.3	4.0	2.6	1.1	1.1	11.0	2.4
301 A	R	3.0	3.5	3.8	2.8	0.8	3.4	1.8	0.0	1.3	0.7	2.6	2.5	50.0	2.6	2.3	2.9	1.8	0.9	9.8	1.8
302 A	V	2.7	4.6	6.7	3.9	2.8	8.9	1.2	6.9	2.7	2.0	2.2	4.8	50.0	4.7	3.2	4.3	7.7	3.8	0.0	8.4
303 A	V	0.0	2.2	3.3	1.0	6.7	4.5	5.3	1.4	2.5	3.1	2.0	3.1	1.0	2.1	2.9	0.4	0.4	2.9	10.9	6.2
304 A	S	0.0	12.1	10.8	20.0	20.0	6.2	20.0	20.0	17.2	20.0	11.9	16.6	50.0	20.0	16.6	2.2	14.2	17.9	20.0	20.0
305 A	V	1.1	2.3	3.3	1.2	0.3	5.4	1.2	0.0	0.9	1.1	2.8	1.1	3.9	1.1	1.4	1.2	0.9	0.0	0.8	0.8

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Table 55 (continued)

Pos	WT	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
306 A	L	4.3	6.2	7.1	5.9	2.8	10.4	3.4	13.7	3.0	0.0	3.5	6.0	50.0	5.9	9.9	6.2	5.3	11.4	9.6	10.3
307 A	T	1.4	3.2	3.8	2.2	6.5	5.5	4.2	0.5	0.3	4.2	3.0	2.2	0.0	1.9	1.3	1.4	0.9	1.2	6.2	6.5
308 A	V	1.8	5.5	6.5	8.0	50.0	7.9	20.0	4.5	20.0	5.5	19.4	7.6	50.0	7.7	15.5	0.0	0.7	5.9	50.0	50.0
309 A	L	1.1	2.7	0.7	0.7	1.3	4.6	2.7	0.7	1.7	1.0	2.8	0.0	1.6	0.7	1.3	1.0	0.6	0.5	5.0	2.1
310 A	H	2.0	2.6	0.9	4.1	50.0	5.6	0.2	6.8	4.0	7.1	4.0	0.0	0.2	4.9	10.0	2.0	2.5	6.4	50.0	50.0
311 A	Q	0.6	2.5	1.6	1.6	2.5	4.3	1.6	1.4	0.6	0.9	2.9	0.9	1.7	0.8	0.9	0.0	0.3	2.2	4.6	2.0
312 A	N	5.4	5.1	5.9	1.3	20.0	0.0	20.0	10.0	3.4	4.8	3.3	7.1	50.0	2.7	3.9	4.1	3.2	11.9	20.0	20.0
313 A	W	4.6	6.4	5.5	5.6	1.1	10.8	5.0	11.0	5.8	5.2	7.6	5.4	50.0	4.8	12.9	6.0	3.8	6.6	0.0	2.6
314 A	L	2.1	2.9	4.3	2.2	5.7	6.1	7.9	5.4	0.7	0.0	1.7	2.3	50.0	1.6	1.6	3.0	4.7	6.3	8.0	6.0
315 A	D	0.3	1.4	1.5	0.1	3.3	4.2	1.9	1.8	0.8	0.5	1.8	0.6	50.0	0.0	0.7	0.0	0.9	2.4	6.2	3.7
316 A	G	50.0	50.0	50.0	50.0	50.0	0.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
317 A	K	0.0	14.0	18.4	17.9	50.0	5.0	50.0	20.0	8.5	12.5	12.7	20.0	15.9	17.2	13.5	2.8	9.2	20.0	50.0	50.0
318 A	E	2.0	3.0	2.7	1.7	2.7	6.7	2.6	0.0	1.1	1.6	1.3	1.7	20.0	1.4	2.6	2.2	1.3	0.0	6.1	9.5
319 A	Y	2.9	4.4	3.9	3.4	0.0	8.8	1.8	20.0	0.5	5.2	0.7	3.2	50.0	3.1	5.6	3.4	3.6	20.0	20.0	0.2
320 A	K	2.3	3.1	3.0	2.7	20.0	7.8	20.0	9.4	0.0	0.6	2.7	1.3	50.0	2.4	1.9	3.3	3.3	7.2	20.0	20.0
321 A	C	0.0	3.2	20.0	18.8	20.0	6.9	20.0	20.0	20.0	20.0	10.4	20.0	50.0	19.6	20.0	1.5	8.7	18.3	20.0	20.0
322 A	K	2.0	2.5	3.5	2.8	2.7	6.4	2.1	0.2	0.1	1.2	2.7	2.7	50.0	2.1	0.0	2.3	1.6	0.9	14.5	2.8
323	V	1.5	2.8	7.3	11.	20.	8.1	20.	6.0	9.6	20.	4.9	8.5	50.	13.	20.	2.8	1.6	0.0	20.	20.

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A					9	0		0			0			0	6	0				0	0
324 A	S	2.0	2.1	0.6	0.0	1.9	4.9	3.9	1.5	2.8	0.7	1.9	0.9	50.0	0.8	2.9	2.7	1.9	2.1	3.8	2.5
325 A	N	2.8	3.9	8.4	3.0	20.0	8.3	20.0	0.0	7.7	20.0	6.2	1.6	13.4	0.5	20.0	3.1	0.1	1.3	20.0	20.0
326 A	K	1.0	2.7	3.0	1.6	3.7	4.1	3.1	3.2	1.7	2.4	3.7	1.2	0.0	0.6	1.4	1.0	1.9	2.6	5.6	3.6
327 A	A	0.9	2.8	5.8	3.1	20.0	6.3	16.7	14.7	2.8	20.0	2.5	5.3	20.0	1.3	4.1	0.0	5.2	13.7	20.0	20.0
328 A	L	6.0	6.3	7.0	4.1	50.0	8.6	20.0	50.0	5.7	0.0	7.1	6.0	50.0	3.7	8.2	6.6	50.0	50.0	20.0	50.0
329 A	P	1.0	2.5	0.9	0.6	4.0	3.4	3.3	1.7	1.9	2.5	3.6	0.0	0.3	0.7	1.5	0.1	1.1	1.1	6.2	3.6
330 A	A	0.9	2.0	1.3	0.7	3.4	3.8	3.0	2.0	1.4	2.0	3.4	0.0	20.0	0.6	1.2	0.2	0.6	1.9	7.0	3.4
331 A	P	50.0	50.0	50.0	50.0	50.0	0.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
332 A	I	1.9	3.7	4.6	1.7	5.0	7.0	1.9	3.8	1.8	0.0	2.5	3.9	20.0	0.8	2.4	2.3	2.6	4.4	20.0	5.9
333 A	E	0.0	3.1	3.2	0.8	4.1	4.4	4.2	16.9	3.6	2.8	2.8	2.5	1.6	1.3	3.2	1.3	1.4	7.7	4.0	4.8
334 A	K	1.7	2.9	2.5	0.0	1.0	6.1	3.3	1.0	1.5	0.5	3.5	1.5	4.4	0.1	2.7	2.2	0.9	1.3	4.9	1.8
335 A	T	0.5	3.2	4.5	2.7	4.2	4.9	4.1	20.0	2.1	3.1	3.0	1.2	0.0	2.3	2.8	1.4	1.4	7.3	5.1	4.5
336 A	I	1.2	1.6	5.0	1.5	20.0	6.1	16.8	0.7	3.4	7.8	2.5	3.2	20.0	2.8	1.4	0.7	0.6	0.0	20.0	20.0
337 A	S	4.8	4.8	7.5	11.5	10.1	0.0	5.5	50.0	9.9	7.0	7.9	5.0	50.0	11.4	12.7	4.5	2.3	50.0	19.3	10.6
338 A	K	1.0	2.7	2.3	2.2	4.6	5.9	2.4	50.0	0.0	2.1	1.9	1.0	50.0	1.5	0.9	0.7	10.3	50.0	5.4	4.9
339 A	A	1.0	2.5	0.8	1.1	4.4	3.7	3.7	2.1	1.8	2.6	3.6	0.0	0.8	0.6	1.6	0.6	0.9	2.4	6.8	3.8
340 A	K	1.3	2.4	2.3	2.0	1.7	4.1	2.3	1.9	0.0	2.3	1.8	1.0	1.9	0.9	1.3	0.5	0.8	1.7	4.9	2.4

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Table 55 (continued)

Pos	WT	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
232 B	P	1.3	3.2	2.2	2.2	4.1	2.9	3.6	1.8	2.1	2.8	3.9	1.1	0.0	1.1	1.6	0.7	1.4	3.0	6.2	4.1
233 B	E	0.5	2.2	1.7	0.5	2.6	3.7	2.9	4.4	1.4	1.1	3.2	0.6	2.7	0.4	1.6	0.0	1.2	6.9	5.5	2.6
234 B	L	2.9	4.0	4.8	4.9	2.0	6.1	0.8	1.5	0.0	1.9	2.7	2.6	20.0	3.6	1.2	3.1	2.5	3.4	13.4	0.5
235 B	L	0.6	2.3	2.4	0.9	5.7	4.9	1.4	3.7	0.0	0.0	1.9	1.9	17.3	0.6	1.4	0.8	0.7	5.2	7.8	5.3
236 B	G	3.6	2.5	5.1	11.8	6.8	0.0	2.8	20.0	5.0	20.0	4.5	3.5	50.0	5.5	19.9	2.6	20.0	20.0	20.0	14.1
237 B	G	20.0	50.0	50.0	50.0	50.0	0.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
238 B	P	3.5	4.7	8.5	4.2	20.0	9.8	20.0	0.0	5.6	9.6	4.6	8.1	1.3	5.8	20.0	4.9	4.4	1.3	20.0	20.0
239 B	S	1.0	2.5	3.4	2.0	7.2	5.7	3.1	3.1	0.6	0.0	2.0	1.9	50.0	1.7	1.1	1.5	1.5	5.2	20.0	5.2
240 B	V	0.1	2.3	7.0	11.9	20.0	6.5	20.0	8.1	12.7	20.0	12.0	7.6	0.0	11.6	20.0	1.2	1.9	0.8	20.0	20.0
241 B	F	0.0	2.0	1.4	0.8	1.0	4.0	2.0	6.5	1.1	0.6	2.3	0.2	50.0	0.3	1.5	0.1	0.9	5.7	4.1	1.1
242 B	L	2.2	3.3	6.5	6.6	6.9	7.9	4.3	0.0	8.7	3.9	4.8	5.3	0.0	9.1	6.8	2.9	1.1	0.5	20.0	8.7
243 B	F	0.8	2.6	1.9	1.7	0.8	4.9	2.0	3.6	1.2	0.8	2.5	0.0	50.0	1.6	2.7	0.1	1.8	3.9	4.3	1.0
244 B	P	1.1	2.1	4.0	1.1	11.9	3.5	5.4	20.0	1.4	3.2	3.0	2.0	1.8	1.2	1.3	0.0	19.6	20.0	9.1	11.0
245 B	P	3.2	20.0	20.0	20.0	20.0	8.6	20.0	20.0	20.0	20.0	20.0	20.0	0.0	20.0	20.0	6.0	20.0	50.0	20.0	20.0
246 B	K	0.5	2.6	1.4	1.2	2.1	4.4	1.6	0.6	0.9	1.4	2.5	0.2	0.3	0.2	0.3	0.1	0.0	2.0	4.9	2.4
247 B	P	0.8	2.5	0.7	1.0	3.6	3.9	2.6	6.2	1.8	2.1	2.9	0.3	0.0	0.8	1.5	0.3	0.7	9.5	6.6	3.4
248 B	K	0.2	2.2	0.2	0.6	2.2	4.1	2.5	2.4	1.7	1.0	2.2	0.0	1.3	0.8	1.7	0.5	0.7	2.8	4.7	2.3
249	D	2.8	3.3	0.0	4.6	10.	8.2	6.5	50.	4.6	6.2	4.4	0.5	50.	4.7	6.3	3.5	6.1	50.	20.	7.2

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B					1			0					0				0	0	
250 B	T	0.0	2.2	4.9	2.8	20.0	6.3	20.0	2.2	4.3	3.2	3.0	9.2	50.0	3.4	4.9	1.3	2.3	3.1	20.0	20.0
251 B	L	0.0	2.4	1.6	1.2	5.6	3.6	2.2	7.4	1.2	0.6	2.3	0.5	50.0	0.6	1.8	0.4	2.5	8.7	8.2	5.9
252 B	M	1.3	2.4	0.8	0.0	1.8	5.7	2.3	0.6	1.6	0.6	2.5	1.0	50.0	1.0	1.6	1.5	1.3	0.8	5.1	1.6
253 B	I	1.6	3.0	2.0	1.2	3.7	4.5	3.5	2.9	0.8	2.4	2.4	1.1	1.0	0.0	1.5	1.2	1.4	3.4	4.4	3.6
254 B	S	1.0	1.5	0.8	0.6	3.8	3.8	3.2	0.5	1.9	2.5	3.1	0.3	6.2	0.5	1.7	0.0	0.1	1.1	5.5	3.7
255 B	R	0.9	2.0	2.0	1.7	0.0	5.4	1.4	20.0	1.0	1.6	1.3	0.8	50.0	1.4	1.1	1.5	20.0	20.0	3.7	0.8
256 B	T	0.6	2.0	1.8	1.1	2.5	3.7	1.9	1.6	1.0	1.4	2.2	0.0	1.2	0.5	0.1	0.8	1.2	1.2	5.5	2.4
257 B	P	2.5	20.0	20.0	20.0	50.0	9.0	50.0	20.0	20.0	20.0	20.0	20.0	0.0	20.0	20.0	4.8	20.0	20.0	50.0	50.0
258 B	E	1.5	2.4	2.7	1.4	2.7	6.4	4.2	0.0	0.2	5.4	2.4	1.1	50.0	1.3	2.5	2.2	1.1	1.0	19.1	3.0
259 B	V	2.9	4.2	6.3	5.2	20.0	9.3	20.0	0.0	8.1	8.9	5.5	5.6	50.0	6.2	20.0	4.5	2.5	0.0	20.0	20.0
260 B	T	0.0	1.6	5.3	1.9	20.0	4.9	20.0	0.6	1.1	2.8	1.4	3.9	0.2	2.3	2.6	0.4	0.1	2.7	20.0	20.0
261 B	C	0.0	10.00	20.0	20.0	20.0	2.6	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	2.0	16.66	20.0	20.0	20.0
262 B	V	2.1	2.4	2.7	2.4	8.1	7.2	3.8	1.8	3.5	8.6	3.4	2.7	50.0	3.2	4.8	2.9	1.9	0.0	14.7	9.1
263 B	V	2.2	3.7	4.7	11.2	20.0	9.1	20.0	15.0	13.7	2.8	20.0	5.4	50.0	13.0	20.0	3.6	2.1	0.0	20.0	20.0
264 B	V	2.1	3.0	4.6	2.7	8.6	6.8	6.6	0.0	1.8	1.8	3.7	3.6	10.1	3.0	2.2	2.6	2.2	1.0	12.7	20.0
265 B	D	4.5	5.2	4.8	4.7	5.0	0.0	3.8	8.5	1.8	2.6	4.1	1.8	50.0	4.5	5.3	4.5	6.0	9.2	12.2	5.6
266 B	V	5.3	5.5	7.2	12.70	20.0	12.00	20.0	2.1	20.0	20.0	20.0	5.7	50.0	18.33	20.0	5.9	4.7	0.0	50.0	50.0
267 B	S	2.8	4.3	6.2	3.8	0.0	7.4	1.0	50.0	1.0	0.3	3.2	3.2	0.5	1.5	0.8	3.3	11.66	50.0	6.3	50.0

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Table 55 (continued)

Pos	WT	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
268 B	H	2.6	3.7	5.1	4.1	4.9	6.0	1.8	2.6	0.0	2.5	3.8	2.6	3.4	2.1	1.8	2.5	3.8	2.7	7.8	5.5
269 B	E	0.4	2.4	1.7	0.8	2.8	3.7	2.6	1.0	1.0	1.6	3.0	0.0	12.8	0.5	0.7	0.3	0.7	0.6	5.1	2.7
270 B	D	0.0	1.6	1.1	7.3	4.8	4.3	2.6	20.0	3.8	14.5	3.8	1.2	5.9	6.3	2.1	0.3	1.9	5.4	16.3	5.6
271 B	P	1.1	3.3	5.6	3.4	4.1	5.5	4.2	20.0	1.9	3.6	3.9	3.3	7.4	2.7	0.0	1.5	2.2	5.2	4.8	4.4
272 B	Q	0.9	1.9	1.0	0.6	3.0	3.9	2.9	1.5	1.7	2.2	3.5	0.6	4.9	0.0	1.4	0.2	0.6	1.4	3.9	3.2
273 B	V	3.5	4.8	6.2	8.3	20.0	9.2	20.0	4.6	8.4	3.1	3.5	7.4	50.0	10.6	20.0	2.0	0.0	4.8	20.0	20.0
274 B	K	0.1	1.6	0.4	0.9	1.7	3.8	1.8	1.9	0.4	0.5	2.4	0.3	15.6	0.1	0.0	0.0	0.2	1.6	2.2	1.9
275 B	F	5.7	7.0	8.4	9.2	0.0	11.2	3.5	9.2	7.9	5.7	5.1	7.0	4.1	9.7	12.3	6.9	4.5	3.3	10.3	5.0
276 B	N	0.0	6.2	6.9	6.4	20.0	4.7	12.1	20.0	9.3	10.0	7.4	3.8	50.0	6.4	9.2	2.8	20.0	20.0	20.0	20.0
277 B	W	8.3	10.0	10.6	9.2	2.6	14.2	7.4	12.7	6.7	7.4	6.4	10.8	6.8	9.3	11.9	9.7	8.0	14.4	0.0	15.9
278 B	Y	0.0	2.3	17.4	4.0	50.0	5.1	50.0	20.0	2.8	20.0	2.1	12.6	11.0	4.4	2.0	0.8	2.5	19.8	20.0	4.2
279 B	V	3.1	3.5	4.2	2.9	20.0	8.5	13.9	0.4	0.0	2.9	2.0	3.4	20.0	1.4	4.0	4.2	2.4	1.2	20.0	20.0
280 B	D	0.5	3.0	2.1	1.5	6.7	3.1	4.7	12.6	2.9	1.6	2.9	1.6	20.0	1.4	3.1	0.0	2.7	5.5	8.1	7.3
281 B	G	5.6	5.8	5.5	4.8	7.9	0.0	7.2	6.5	5.3	5.7	7.1	3.4	50.0	4.0	5.3	3.6	3.2	6.4	10.3	7.6
282 B	V	0.4	1.9	1.1	0.6	2.9	4.1	2.1	1.3	1.0	1.4	2.9	0.2	50.0	0.0	0.7	0.0	0.4	0.7	6.1	2.8
283 B	E	0.6	1.9	4.3	1.7	6.7	4.2	5.2	2.9	0.5	4.4	0.3	2.5	0.0	1.5	1.6	1.0	1.5	3.9	7.9	6.7
284 B	V	0.4	2.4	2.5	1.1	20.0	5.9	20.0	1.1	1.2	6.2	0.8	2.4	50.0	1.5	3.3	0.0	1.5	1.8	20.0	20.0
285	H	1.3	2.4	2.1	1.7	2.4	3.4	1.2	1.8	0.7	2.3	2.7	0.0	1.6	0.9	0.8	0.5	0.4	2.0	5.8	2.4

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B																								
286 B	N	1.2	2.7	1.0	1.1	3.0	3.1	2.6	0.8	2.0	1.9	2.9	0.0	50.0	0.4	1.6	1.1	0.5	2.9	4.9	3.0
287 B	A	2.5	4.4	6.1	7.5	0.0	8.2	3.0	10.2	5.1	16.5	4.5	0.3	12.3	8.1	9.1	4.1	3.3	7.1	3.4	0.8
288 B	K	0.4	1.9	1.9	0.0	2.9	3.5	2.9	2.5	1.8	2.1	2.5	0.9	15.4	0.6	1.1	0.2	0.9	3.8	5.9	2.7
289 B	T	0.1	1.5	3.7	1.4	2.7	3.9	2.6	1.8	0.0	2.2	1.6	1.5	1.8	1.1	1.7	0.0	0.4	2.3	3.4	2.5
290 B	K	0.9	1.8	0.8	0.5	2.4	0.8	2.7	3.0	1.3	1.3	2.6	0.2	50.0	0.7	1.5	0.0	0.6	2.9	5.0	2.7
291 B	P	1.2	2.1	2.5	0.5	3.9	4.6	3.4	0.7	0.0	3.4	1.5	1.1	0.6	0.1	1.1	1.1	0.9	1.5	3.2	2.6
292 B	R	0.8	2.6	3.3	1.2	4.9	3.6	6.8	3.1	2.0	2.4	2.2	2.2	16.6	1.5	1.8	0.1	0.0	3.2	7.6	5.2
293 B	E	0.0	3.0	4.1	2.8	7.3	3.6	5.8	5.8	2.6	4.5	3.2	2.2	1.3	2.2	2.5	0.0	1.2	7.8	7.0	6.9
294 B	E	2.5	3.3	3.9	2.3	8.3	6.8	4.4	5.6	3.6	2.3	3.7	4.1	0.0	3.3	5.0	2.1	2.9	5.0	6.7	11.9
295 B	Q	1.1	2.2	1.9	0.6	3.8	2.8	3.1	8.0	1.4	2.2	3.4	1.0	0.4	0.4	1.1	0.0	3.9	6.6	6.1	3.5
296 B	Y	1.5	2.7	1.2	1.2	4.1	4.1	3.5	1.1	1.8	2.7	3.5	0.0	20.0	0.6	1.9	1.2	1.4	1.3	6.4	4.0
297 B	N	3.9	4.5	10.1	6.0	15.5	7.3	16.7	6.6	0.0	5.1	4.6	7.3	20.0	4.4	4.2	3.6	4.1	7.9	18.0	15.0
298 B	S	1.7	2.5	3.5	2.5	2.5	3.7	2.4	3.0	0.0	1.8	2.3	0.4	50.0	1.2	1.0	0.9	2.2	3.3	5.5	2.0
299 B	T	0.0	2.7	7.2	11.1	20.0	4.8	20.0	7.5	6.9	20.0	7.1	4.8	50.0	9.8	17.9	0.3	1.3	5.8	20.0	20.0
300 B	Y	3.8	5.2	8.0	4.3	20.0	8.6	20.0	12.2	0.0	4.3	3.2	6.5	50.0	4.0	3.8	4.3	3.6	9.1	20.0	6.4
301 B	R	1.2	1.8	2.3	1.1	20.0	5.8	11.3	5.2	0.3	5.0	2.0	1.6	14.1	0.6	0.4	1.8	1.1	0.0	17.9	20.0
302 B	V	3.5	4.8	5.5	3.7	0.2	9.6	1.1	0.5	2.6	3.5	2.5	4.7	9.6	4.1	0.6	4.3	2.0	0.0	20.0	0.2
303 B	V	0.2	0.0	0.1	1.0	20.0	5.0	13.3	5.1	1.7	10.4	1.9	2.0	8.6	2.0	4.7	0.6	0.5	1.3	20.0	20.0
304	S	1.5	2.3	8.2	20.	20.	7.6	20.	7.6	20.	20.	20.	6.3	50.	20.	20.	0.0	2.7	3.8	20.	20.

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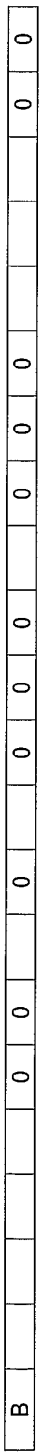

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Table 55 (continued)

Pos	WT	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
304 B	S	1.5	2.3	8.2	20.0	20.0	7.6	20.0	7.6	20.0	20.0	20.0	6.3	50.0	20.0	20.0	0.0	2.7	3.8	20.0	20.0
305 B	V	0.1	1.2	3.3	1.1	20.0	4.6	20.0	3.2	1.1	11.0	1.5	1.8	50.0	0.6	2.0	0.6	0.0	0.7	20.0	20.0
306 B	L	4.7	6.8	6.3	4.3	10.4	11.1	7.8	4.2	3.0	0.0	3.8	5.7	13.4	4.4	14.1	5.5	4.3	6.0	20.0	12.1
307 B	T	1.5	3.0	2.7	1.7	4.1	5.2	3.0	1.6	1.9	3.1	3.4	1.7	0.0	1.7	1.9	1.5	1.4	2.0	4.4	4.3
308 B	V	0.0	0.6	7.6	20.0	20.0	6.6	20.0	20.0	16.1	15.1	20.0	12.4	50.0	20.0	20.0	1.2	3.6	4.3	20.0	20.0
309 B	L	1.4	3.0	2.2	1.1	3.0	6.0	3.5	20.0	2.4	1.7	3.6	0.2	0.0	1.6	2.3	1.8	14.3	20.0	5.1	3.3
310 B	H	2.4	2.9	2.7	4.9	20.0	6.8	4.4	4.8	3.1	15.0	3.4	0.0	2.3	4.6	7.0	1.8	1.6	3.8	20.0	20.0
311 B	Q	0.0	2.2	1.3	0.7	2.1	3.3	2.4	12.6	0.6	0.9	2.3	0.6	3.2	0.4	0.8	0.2	1.6	18.8	4.6	2.0
312 B	N	0.0	1.0	0.2	0.3	6.0	5.4	2.3	12.0	2.1	2.9	1.6	0.9	50.0	1.3	5.7	0.1	5.6	3.8	8.0	7.8
313 B	W	5.3	6.6	7.3	5.4	0.0	11.4	6.2	20.0	4.0	5.2	4.3	8.0	50.0	6.5	8.9	6.6	17.2	20.0	2.1	0.9
314 B	L	1.7	2.2	3.1	0.0	6.4	5.6	1.5	2.1	0.6	0.2	1.1	1.9	50.0	0.8	1.0	1.7	0.9	3.1	3.7	11.3
315 B	D	1.4	2.3	2.4	0.7	6.0	5.5	2.3	4.8	2.2	1.0	2.9	1.8	50.0	1.0	2.2	0.2	0.0	4.5	8.5	6.8
316 B	G	50.0	50.0	50.0	50.0	50.0	0.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
317 B	K	0.9	2.3	4.3	2.8	1.2	4.0	0.6	13.9	0.0	4.8	1.6	1.3	50.0	4.2	0.9	0.4	13.8	10.1	20.0	1.7
318 B	E	0.7	1.2	3.1	1.0	7.0	5.1	8.2	0.4	1.0	5.7	1.7	2.3	3.8	1.0	1.6	0.4	0.0	1.0	3.8	7.7
319 B	Y	6.5	7.1	8.5	8.8	0.0	12.5	3.9	3.1	5.2	5.4	8.4	7.2	50.0	9.0	13.7	7.2	5.8	3.9	20.0	1.7
320 B	K	3.1	4.3	7.3	4.3	20.0	8.6	15.0	1.4	0.0	11.6	3.6	6.6	50.0	2.9	2.4	4.0	3.3	2.0	20.0	20.0
321	C	0.0	6.5	20.	20.	20.	6.6	20.	20.	20.	20.	20.	20.	19.	20.	20.	3.1	11.	20.	20.	20.

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B				0	0	0		0	0	0	0	0	7	0	0		2	0	0	0
322 B	K	2.3	3.2	3.5	1.8	20.0	7.9	20.0	1.1	0.6	4.9	3.7	2.2	50.0	0.9	0.3	3.3	1.6	0.0	20.0	20.0
323 B	V	4.0	4.6	6.9	8.1	20.0	10.6	20.0	9.0	17.1	7.9	8.1	10.5	50.0	8.7	20.0	5.6	4.6	0.0	20.0	20.0
324 B	S	1.3	3.0	1.4	0.0	2.1	6.0	4.4	1.3	2.4	0.6	2.7	2.2	50.0	1.3	3.6	1.5	1.6	1.8	3.6	2.4
325 B	N	3.4	5.1	9.0	4.7	20.0	8.2	20.0	16.6	16.6	20.0	20.0	0.0	50.0	6.3	20.0	4.6	8.8	17.8	20.0	20.0
326 B	K	0.3	2.1	2.0	0.9	1.0	3.5	2.0	2.9	0.9	2.9	2.8	0.1	4.4	0.0	1.1	0.1	3.2	2.1	5.2	0.7
327 B	A	1.9	3.3	4.7	3.5	20.0	7.0	20.0	20.0	0.3	0.0	1.9	1.9	20.0	3.0	2.3	3.3	20.0	20.0	20.0	20.0
328 B	L	3.7	3.6	3.8	4.4	50.0	8.4	7.0	50.0	3.8	0.0	2.6	4.0	50.0	4.2	8.7	4.8	2.9	12.3	50.0	50.0
329 B	P	3.3	8.5	20.0	20.0	50.0	8.0	16.5	50.0	18.5	20.0	14.7	20.0	0.0	20.0	20.0	1.4	17.1	16.4	50.0	50.0
330 B	A	0.5	2.0	2.8	0.5	2.4	3.9	1.2	4.0	0.0	2.0	2.1	0.8	20.0	0.0	0.5	0.8	0.2	4.6	8.2	2.6
331 B	P	1.7	3.8	6.4	10.1	20.0	4.7	11.0	10.1	7.5	20.0	5.5	5.0	0.0	7.6	7.4	2.6	20.0	10.1	17.6	20.0
332 B	I	1.7	2.9	1.3	1.7	14.8	7.0	13.9	1.7	3.1	0.0	1.7	1.7	50.0	1.8	5.3	2.0	1.9	3.4	20.0	20.0
333 B	E	1.9	2.5	1.9	0.0	8.9	5.9	8.2	1.2	3.0	6.4	3.4	2.0	3.1	1.1	2.3	1.8	1.6	1.6	8.9	9.3
334 B	K	2.9	3.9	3.7	2.6	20.0	8.3	12.1	1.5	2.6	5.3	3.7	4.3	50.0	1.9	0.0	3.4	1.8	1.4	9.9	20.0
335 B	T	0.0	2.1	7.2	7.0	4.2	0.4	3.3	17.3	6.5	7.7	5.2	5.5	3.5	7.0	5.7	0.2	5.5	11.5	5.2	3.1
336 B	I	0.5	1.6	2.1	0.7	20.0	5.0	6.1	0.0	1.3	5.3	2.1	1.8	20.0	0.6	3.1	1.1	0.8	0.7	19.4	20.0
337 B	S	1.1	2.1	4.0	2.0	3.1	3.2	2.0	50.0	0.0	1.6	0.9	1.9	15.8	1.1	2.2	1.4	50.0	50.0	5.5	3.9
338 B	K	0.6	2.3	3.0	3.0	9.4	5.3	10.6	2.2	1.1	0.0	3.2	1.5	16.2	2.7	2.7	1.1	2.8	3.5	8.1	11.0
339 B	A	1.1	2.4	1.2	0.8	4.3	3.6	3.7	2.6	1.8	2.6	3.5	0.0	2.3	0.5	1.3	0.2	0.8	2.0	6.7	3.8
340	K	0.9	2.0	1.4	0.8	3.0	3.4	2.9	2.1	0.8	2.5	2.3	0.2	1.0	0.1	1.2	0.0	0.5	2.0	5.5	3.2

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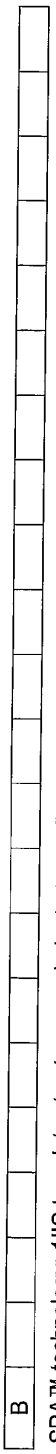

SPA™ technology; 1IIS template structure; - carbohydrate, no floated positions

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Table 56

Pos	WT	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
239 A	S	0.2	4.6	2.7	0.0	20.0	4.6	14.5	11.0	1.9	0.3	2.0	1.9	8.1	1.4	2.6	0.4	5.7	11.6	20.0	20.0
240 A	V	1.5	2.4	2.4	6.9	20.0	7.4	20.0	5.1	9.9	5.9	5.5	2.4	1.1	12.3	13.1	2.6	0.5	0.0	20.0	20.0
263 A	V	2.3	2.8	6.3	16.5	20.0	8.8	20.0	9.6	7.3	7.3	15.3	4.8	50.0	16.4	17.4	2.8	1.4	0.0	20.0	20.0
264 A	V	1.8	3.1	2.6	1.8	0.0	6.3	1.9	0.6	2.4	0.8	2.7	2.1	1.6	2.3	2.7	2.3	1.1	0.5	3.5	0.0
266 A	V	4.9	5.2	6.9	12.3	20.0	11.1	20.0	0.8	11.9	20.0	8.5	6.6	50.0	12.5	20.0	6.1	3.7	0.0	20.0	20.0
296 A	Y	3.4	2.7	1.1	0.0	50.0	0.7	50.0	5.0	3.6	3.5	4.2	0.9	50.0	0.9	2.9	2.2	5.3	5.5	16.1	18.4
299 A	T	0.7	3.2	9.9	10.4	20.0	6.2	20.0	10.7	6.7	20.0	4.1	12.9	50.0	5.9	11.8	0.0	2.5	8.2	13.3	20.0
325 A	N	2.5	3.5	7.7	2.5	20.0	8.0	20.0	0.0	6.1	20.0	7.8	1.2	12.8	0.8	20.0	2.7	0.0	1.0	20.0	20.0
328 A	L	6.1	6.3	7.1	4.2	50.0	8.8	20.0	50.0	4.6	0.0	7.2	6.1	50.0	4.0	8.3	6.7	50.0	50.0	20.0	50.0
330 A	A	0.9	1.8	1.2	0.0	2.5	4.0	2.9	1.7	1.2	1.6	2.8	0.0	20.0	0.4	1.0	0.2	0.5	1.7	6.2	2.9
332 A	I	1.9	3.8	4.6	1.3	5.1	7.1	1.8	3.4	0.2	0.0	2.6	3.8	20.0	0.6	2.4	2.3	2.5	4.2	20.0	5.6
239 B	S	1.0	2.4	3.5	2.0	6.7	5.6	2.9	3.1	0.3	0.0	1.9	2.1	50.0	1.5	1.8	1.4	1.4	5.2	20.0	4.2
240 B	V	0.3	2.4	6.9	11.7	20.0	6.6	20.0	8.3	12.3	20.0	14.2	7.4	0.0	13.4	20.0	1.3	1.9	0.9	20.0	20.0
263 B	V	2.4	3.9	4.5	12.5	20.0	9.3	20.0	15.8	17.1	2.1	20.0	5.3	50.0	13.8	20.0	3.9	2.2	0.0	20.0	20.0
264 B	V	2.2	3.2	4.8	2.7	7.4	6.9	6.0	0.0	1.9	1.9	3.8	3.7	9.9	3.1	2.2	2.7	2.4	0.9	14.7	18.2
266 B	V	5.4	5.5	7.5	13.2	20.0	12.1	20.0	2.6	20.0	20.0	20.0	5.4	50.0	16.1	20.0	6.0	4.7	0.0	50.0	50.0
296 B	Y	1.5	2.7	1.3	1.2	4.0	4.1	3.6	1.1	1.9	2.6	3.5	0.0	20.0	0.7	1.8	1.1	1.4	1.3	6.5	4.2
299	T	0.0	2.2	7.5	10.	20.	4.8	20.	7.7	5.8	20.	10.	5.1	50.	10.	18.	0.3	1.1	5.4	20.	20.

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B					2	0		0			0	3		0	2	4			0	0
325 B	N	3.4	5.1	8.6	5.0	20.0	8.2	20.0	16.7	20.0	20.0	20.0	0.0	19.7	6.3	20.0	4.6	8.6	18.2	20.0	20.0
328 B	L	3.6	3.5	3.8	3.9	50.0	8.3	7.0	50.0	2.9	0.0	1.9	3.8	50.0	3.4	8.4	4.7	2.9	12.5	50.0	50.0
330 B	A	0.7	2.1	2.9	0.7	2.7	4.0	1.4	4.8	0.0	2.2	2.3	0.8	20.0	0.2	0.8	1.1	0.2	4.7	7.8	3.2
332 B	I	1.8	2.9	1.2	1.8	13.5	7.0	9.9	1.7	3.2	0.0	1.7	1.9	50.0	1.2	5.4	2.0	2.0	3.3	20.0	20.0

SPA™ technology; D129G 1IIS template structure; + carbohydrate

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Table 57

Pos	WT	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
239 A	S	1.2	3.5	1.7	0.0	20.0	5.8	11.0	6.6	2.9	3.9	3.9	2.7	8.5	1.3	2.7	0.6	3.5	5.4	20.0	20.0
240 A	V	1.2	2.4	6.0	14.0	20.0	7.1	20.0	6.7	9.4	10.1	7.5	4.4	1.8	14.8	20.0	2.0	0.4	0.0	20.0	20.0
263 A	V	0.0	0.4	1.0	8.7	20.0	6.9	4.4	11.7	4.9	16.0	19.2	0.8	50.0	11.7	20.0	1.4	0.1	1.0	20.0	20.0
264 A	V	2.9	3.7	6.3	2.8	11.6	7.6	13.2	0.0	3.2	3.4	4.1	4.2	7.1	2.9	3.4	3.1	1.9	0.8	12.8	16.3
266 A	V	4.8	5.9	6.8	9.5	50.0	10.3	20.0	3.5	12.7	12.2	12.7	4.1	50.0	11.9	11.9	5.2	2.9	0.0	50.0	50.0
296 A	Y	0.8	2.0	1.5	0.1	0.2	3.4	1.5	6.6	1.7	0.6	1.8	1.2	2.6	0.0	1.6	0.2	2.5	5.6	3.8	0.0
299 A	T	1.9	3.7	7.5	0.0	20.0	7.9	14.2	2.9	0.8	3.4	4.4	2.3	50.0	1.9	3.0	3.5	4.1	3.3	20.0	20.0
325 A	N	1.0	1.4	3.1	2.8	20.0	7.4	20.0	8.5	7.7	10.4	6.1	2.8	15.4	5.4	20.0	0.0	0.1	3.8	20.0	20.0
328 A	L	2.5	5.3	4.0	1.9	50.0	7.5	20.0	20.0	1.6	0.2	0.0	2.9	50.0	0.4	4.8	3.2	2.9	7.0	50.0	50.0
330 A	A	0.9	2.1	1.8	1.2	2.4	2.7	3.1	3.1	1.4	2.1	3.5	0.5	20.0	0.8	1.0	0.0	0.5	2.9	5.2	2.9
332 A	I	2.9	3.7	3.9	0.9	6.1	7.8	2.5	0.0	2.7	0.8	2.8	3.5	50.0	0.7	3.7	2.9	2.5	1.0	8.1	6.9
239 B	S	1.9	3.1	3.0	1.9	1.5	6.2	2.3	14.1	1.8	1.4	2.9	1.8	0.0	1.9	3.2	1.9	2.3	7.7	6.6	15.8
240 B	V	0.5	1.7	5.0	13.3	20.0	6.6	20.0	1.2	12.4	12.1	8.8	4.6	6.3	20.0	20.0	1.0	0.2	0.0	20.0	20.0
263 B	V	2.9	3.2	6.4	18.2	10.1	9.2	6.9	12.8	6.0	20.0	10.3	5.7	50.0	17.5	20.0	3.2	2.2	0.0	20.0	20.0
264 B	V	2.9	3.6	4.4	3.0	8.8	7.1	6.2	0.0	2.3	1.9	4.5	3.4	1.7	3.2	3.5	3.5	2.0	0.9	12.0	16.4
266 B	V	4.4	4.6	2.6	6.6	20.0	10.7	20.0	0.0	4.9	1.7	8.5	5.6	50.0	6.0	12.4	5.3	4.6	1.5	20.0	50.0
296 B	Y	0.0	7.1	6.7	7.2	20.0	0.1	18.6	50.0	7.0	2.7	6.6	6.8	50.0	7.2	9.3	2.3	50.0	50.0	20.0	14.1
299	T	0.0	3.2	10.	6.0	20.	5.5	20.	15.	3.2	5.9	4.4	6.4	50.	5.7	9.4	1.2	1.4	13.	20.	20.

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B				4		0		0	9					0				7	0	0
325 B	N	1.4	2.5	5.0	0.0	20.0	7.0	20.0	20.0	1.0	2.2	1.0	0.3	1.9	1.1	20.0	2.6	5.1	20.0	20.0
328 B	L	0.4	1.3	5.6	0.0	50.0	4.5	50.0	50.0	1.9	2.4	2.4	8.3	50.0	0.8	16.4	1.0	1.2	50.0	50.0
330 B	A	0.6	1.4	2.5	0.9	3.1	2.5	1.2	20.0	0.0	2.4	2.1	0.3	20.0	0.4	0.6	0.0	4.0	20.0	13.5	3.4
332 B	I	4.3	5.3	5.7	0.0	11.4	9.3	4.3	2.5	5.8	2.0	4.0	6.5	17.9	3.7	5.9	4.6	4.2	3.7	20.0	11.6

SPA™ technology; D129G 1IIX template structure; + carbohydrate

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Table 58

Pos	WT	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
239 A	S	1.2	2.3	2.2	1.8	7.9	5.5	7.6	0.5	0.2	1.8	2.6	1.4	0.9	1.3	1.9	1.5	0.8	0.0	8.6	9.6
240 A	V	0.7	2.9	6.8	4.3	20.0	6.5	20.0	0.0	10.7	20.0	3.1	9.1	2.1	7.7	20.0	1.4	1.1	2.4	20.0	20.0
263 A	V	1.7	2.9	4.6	18.8	20.0	8.4	5.8	15.1	2.3	14.5	2.1	3.2	50.0	20.0	15.0	3.6	1.2	0.0	20.0	20.0
264 A	V	2.7	3.3	3.6	1.5	13.9	6.7	5.9	0.0	2.3	4.9	3.7	3.2	1.9	2.5	3.0	3.0	2.5	0.7	19.9	19.0
266 A	V	3.5	3.5	5.7	12.4	20.0	10.0	20.0	5.7	6.3	7.8	7.4	5.2	50.0	16.6	20.0	4.2	1.7	0.0	20.0	50.0
296 A	Y	2.6	50.0	50.0	50.0	50.0	0.0	50.0	50.0	18.5	18.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	13.6
299 A	T	0.2	0.7	6.6	1.2	20.0	5.6	9.6	1.6	0.8	1.5	1.8	4.8	50.0	1.0	9.2	0.0	0.0	1.6	20.0	20.0
325 A	N	3.1	3.6	7.3	2.4	20.0	7.7	20.0	20.0	20.0	10.0	13.1	3.6	50.0	0.0	20.0	4.0	9.7	20.0	20.0	20.0
328 A	L	0.6	0.0	1.5	5.4	50.0	1.6	50.0	50.0	3.1	4.2	9.6	1.4	50.0	6.9	9.6	0.6	0.1	50.0	50.0	50.0
330 A	A	1.9	2.5	4.1	2.8	4.5	4.1	3.0	3.2	1.0	2.7	3.5	2.1	20.0	2.4	2.6	1.3	0.0	3.9	7.6	5.3
332 A	I	2.3	3.5	2.2	0.8	20.0	6.8	9.6	0.0	3.4	0.2	2.6	2.8	14.5	3.3	4.6	2.6	1.3	0.9	10.5	20.0
239 B	S	1.4	3.6	2.5	1.4	16.8	5.8	6.2	5.0	2.5	1.4	2.0	3.8	0.3	0.5	2.4	0.0	1.6	5.3	20.0	19.5
240 B	V	0.0	2.6	12.8	18.6	20.0	5.7	20.0	12.7	10.4	20.0	8.5	15.1	3.1	20.0	20.0	1.0	0.2	2.4	20.0	20.0
263 B	V	1.1	2.4	3.6	20.0	20.0	7.8	17.7	11.8	4.5	20.0	6.3	3.3	50.0	20.0	20.0	3.2	1.2	0.0	20.0	20.0
264 B	V	3.3	4.0	5.0	2.9	14.2	7.5	4.8	0.0	2.6	3.6	4.6	3.5	1.7	3.1	4.1	3.9	2.9	1.3	6.9	20.0
266 B	V	2.9	3.3	4.9	11.3	50.0	9.5	20.0	20.0	20.0	7.9	15.0	4.5	50.0	4.9	20.0	1.9	0.0	3.6	50.0	50.0
296 B	Y	2.8	50.0	50.0	50.0	50.0	0.0	50.0	50.0	17.7	18.7	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	11.3
299	T	0.0	3.8	12.	9.2	20.	5.9	20.	7.3	4.8	3.2	4.3	8.0	50.	12.	8.8	0.2	2.1	4.4	20.	20.

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B				6		0		0						0	3				0	0
325 B	N	0.3	2.0	5.5	2.2	50.0	6.1	20.0	0.0	10.5	15.5	14.6	1.3	10.0	2.4	20.0	2.3	2.0	1.0	20.0	50.0
328 B	L	5.4	5.7	7.3	4.4	50.0	9.8	20.0	50.0	2.5	0.0	5.1	5.9	50.0	2.8	7.4	6.1	6.4	50.0	50.0	50.0
330 B	A	0.6	1.4	3.2	1.3	3.9	3.2	2.7	4.0	1.3	3.7	3.1	0.7	20.0	0.6	1.3	0.0	0.4	4.2	8.2	3.6
332 B	I	1.9	3.1	2.7	1.7	5.2	6.9	3.1	0.4	1.3	0.0	1.9	2.6	7.7	1.3	2.2	2.3	1.6	2.0	10.4	5.6

SPA™ technology; D129G 1E4K template structure; + carbohydrate

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Table 59

Pos	WT	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
239 A	S	1.4	2.6	3.1	1.0	20.0	5.7	4.8	3.4	2.0	1.2	2.6	1.6	4.8	0.0	2.1	1.3	2.1	3.3	13.8	19.6
240 A	V	2.9	3.5	3.7	4.6	20.0	8.2	10.8	0.0	9.1	3.2	5.4	3.1	4.8	5.5	17.5	4.0	1.8	1.2	20.0	20.0
263 A	V	3.6	4.9	6.2	8.7	20.0	9.9	20.0	3.7	4.2	0.5	6.7	6.1	50.0	9.5	20.0	5.1	3.6	0.0	20.0	20.0
264 A	V	1.8	2.8	3.3	2.0	2.9	6.2	3.1	0.0	2.4	0.8	3.0	2.4	6.1	1.4	2.8	2.4	1.9	0.8	10.2	2.2
266 A	V	4.4	5.2	4.9	7.1	20.0	10.6	20.0	1.0	12.1	4.8	9.1	4.6	50.0	7.9	12.6	5.8	3.5	0.0	20.0	20.0
296 A	Y	1.2	2.9	0.7	1.4	3.1	3.9	2.7	2.4	2.3	1.9	2.2	0.0	1.6	1.4	3.0	0.9	1.0	3.5	6.0	2.6
299 A	T	0.0	2.6	6.0	11.5	20.0	5.3	20.0	20.0	6.0	20.0	4.4	3.0	50.0	14.1	13.2	0.9	3.8	15.1	15.0	20.0
325 A	N	5.2	7.0	6.6	6.9	50.0	11.3	20.0	1.3	14.3	13.5	13.9	0.0	5.0	6.0	20.0	6.0	4.6	3.2	20.0	50.0
328 A	L	4.8	5.5	7.0	3.2	20.0	10.5	20.0	50.0	5.1	0.0	8.5	5.5	50.0	3.5	8.2	5.5	13.4	50.0	20.0	50.0
330 A	A	0.9	1.8	1.1	0.9	3.5	4.0	3.0	2.3	1.2	1.6	2.8	0.0	14.5	0.9	1.1	0.1	0.4	2.0	6.4	3.2
332 A	I	5.3	6.4	6.7	4.8	8.2	9.9	5.2	3.1	0.0	3.6	5.2	6.8	20.0	3.5	4.6	5.5	4.8	4.0	11.2	7.1
239 B	S	0.7	2.3	2.6	2.0	5.3	5.1	3.3	1.7	0.0	0.0	2.0	0.8	15.5	0.9	0.8	0.7	0.7	3.3	8.2	6.0
240 B	V	2.3	3.0	4.1	7.3	20.0	8.1	20.0	5.1	20.0	11.8	10.9	3.8	2.0	17.0	20.0	3.6	1.3	0.0	20.0	20.0
263 B	V	3.2	4.3	7.3	8.3	20.0	9.6	20.0	13.3	8.5	0.6	20.0	6.0	50.0	8.5	20.0	4.6	4.0	0.0	20.0	20.0
264 B	V	2.1	3.2	3.7	2.7	17.8	6.6	11.5	0.0	2.0	0.8	3.5	3.0	7.8	2.0	1.5	2.5	1.3	1.0	13.9	20.0
266 B	V	5.0	5.0	5.2	16.3	20.0	11.2	20.0	2.3	20.0	14.3	17.3	2.5	50.0	11.6	20.0	5.4	3.9	0.0	20.0	20.0
296 B	Y	0.9	2.3	1.0	0.5	2.7	3.7	2.5	1.2	1.3	2.1	3.0	0.0	7.0	0.4	1.1	0.3	0.8	1.8	6.0	2.4
299	T	1.1	2.2	7.6	5.4	20.	6.4	12.	1.8	3.9	17.	6.9	3.9	20.	4.6	10.	0.8	0.0	1.9	20.	20.

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B						0		8			5			0		3			0	0
325 B	N	10.1	11.5	13.1	11.2	20.0	15.7	20.0	8.6	14.3	17.1	20.0	0.0	16.1	10.6	20.0	11.1	10.9	10.5	20.0	20.0
328 B	L	2.9	4.1	4.8	3.5	50.0	8.5	1.7	9.6	1.5	0.0	1.5	3.5	50.0	3.3	2.0	3.3	1.9	5.2	50.0	50.0
330 B	A	0.1	2.0	1.4	1.8	1.6	4.0	3.0	2.0	0.5	0.5	2.6	0.0	20.0	0.7	2.0	0.3	0.6	2.1	4.4	2.4
332 B	I	3.4	4.4	3.5	3.1	6.1	8.2	4.1	0.0	3.3	1.3	3.3	4.0	15.7	0.8	2.1	3.9	2.7	1.1	20.0	6.1

SPA™ technology; Fc/Fc□RIIb model template structure; - carbohydrate

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[202] The results of the design calculations presented above in Tables 1 – 59 were used to construct a series of Fc variant libraries for experimental production and screening. Experimental libraries were designed in successive rounds of computational and experimental screening. Design of subsequent Fc libraries benefitted from feedback from prior libraries, and thus typically comprised combinations of Fc variants that showed favorable properties in the previous screen. The entire set of Fc variants that were constructed and experimentally tested is shown in Table 60. In this table, row 1 lists the variable positions, and the rows that follow indicate the amino acids at those variable positions for WT and the Fc variants. For example, variant 18 has the following four mutations: F241E, F243Y, V262T, and V264R. The variable position residues that compose this set of Fc variants are illustrated structurally in Figure 3, and are presented in the context of the human IgG1 Fc sequence in Figure 4.

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Table 60

Position	234	235	239	240	241	243	244	245	247	262	263	264	265	266	267	269	296	297	298	299	313	325	326	327	328	329	330	332	333	334
WT	L	L	S	V	F	F	P	P	P	V	V	V	D	V	S	E	Y	N	S	T	W	N	K	A	L	P	A	I	E	K
1												A																		
2												L																		
3												I																		
4					W																									
5					L																									
6						W																								
7						L																								
8					L	L				I		I																		
9					W	W																								
10					W	W				A		A																		
11					L					I																				
12						L						I																		
13						L				I		W																		
14					Y	Y				T		T																		
15					E	R				E		R																		
16					E	Q				T		E																		
17					R	Q				T		R																		
18					E	Y				T		R																		
19																									M					
20																									E					
21																									F					
22																												E		
23																									M			E		
24							H																							
25								A																						
26									V																					
27																					F									
28							H	A	V																					
29									G																					
30												I																E		

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Table 60 (continued)

Position	234	235	239	240	241	243	244	245	247	262	263	264	265	266	267	269	296	297	298	299	313	325	326	327	328	329	330	332	333	334
WT	L	L	S	V	F	F	P	P	P	V	V	V	D	V	S	E	Y	N	S	T	W	N	K	A	L	P	A	I	E	K
31					E	R				E		R																E		
32					E	Q				T		E																E		
33					R	Q				T		R																E		
34					E	Y				T		R																E		
35																			A											
36																			A								E			
37																			A									A	A	
41			E																								E			
42			Q																								E			
43			E																											
44													G																	
45													N																	
46			E										G																	
47			E										N																	
48			E										Q																	
49																	E													
50																	Q													
51																			T											
52																			N											
53																				I										
54																								S						
55																								N						
56															Q									S						
57															L									S						
58																								L						
59																										F				
60																											L			
61																											Y			
62																												D		

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Table 60 (continued)

Position	234	235	239	240	241	243	244	245	247	262	263	264	265	266	267	269	296	297	298	299	313	325	326	327	328	329	330	332	333	334
WT	L	L	S	V	F	F	P	P	P	V	V	V	D	V	S	E	Y	N	S	T	W	N	K	A	L	P	A	I	E	K
62																												D		
63																		S												
64																		D												
65																		S										E		
66																		D										E		
67																		E										E		
68													Y					D										E		
69													Y					D										E		
70													F					E										E		
71																									I			E		
72																									Q			E		
73																												N		
74																												Q		
75												T																		
76												F																		
77				I																										
78											I																			
79														I																
80																				A										
81																				S										
82																				V										
83																						Q								
84																						L								
85																						I								
86			D																											
87			N																											
88			F																											
89			D																									D		
90			D																									E		
91			D																									N		
92			D																									Q		

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Table 60 (continued)

Position	234	235	239	240	241	243	244	245	247	262	263	264	265	266	267	269	296	297	298	299	313	325	326	327	328	329	330	332	333	334
WT	L	L	S	V	F	F	P	P	P	V	V	V	D	V	S	E	Y	N	S	T	W	N	K	A	L	P	A	I	E	K
93			E																									D		
94			E																									N		
95			E																									Q		
96			N																									D		
97			N																									E		
98			N																									N		
99			N																									Q		
100			Q																									D		
101			Q																									N		
102			Q																									Q		
103																						E								
104																	D													
105																	N													
106					Y	Y				T		T						D										E		
107																											Y	E		
108												I															Y	E		
109																											L	E		
110												I															L	E		
111	D																													
112	E																													
112	N																													
114	Q																													
115	T																													
116	H																													
117	Y																													
118	I																													
119	V																													
120	F																													
121		D																												
122		S																												

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Table 60 (continued)

Position	234	235	239	240	241	243	244	245	247	262	263	264	265	266	267	269	296	297	298	299	313	325	326	327	328	329	330	332	333	334
WT	L	L	S	V	F	F	P	P	P	V	V	V	D	V	S	E	Y	N	S	T	W	N	K	A	L	P	A	I	E	K
123		N																												
124		Q																												
125		T																												
126		H																												
127		Y																												
128		I																												
129		V																												
130		F																												
131			T																											
132			H																											
133			Y																											
134				A																										
135				T																										
136				M																										
137											A																			
138											T																			
139											M																			
140												M																		
141												Y																		
142														A																
143														T																
144														M																
145																H														
146																Y														
147																F														
148																R														
149																	S													
150																	T													
151																	L													
152																	I													

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Table 60 (continued)

Position	234	235	239	240	241	243	244	245	247	262	263	264	265	266	267	269	296	297	298	299	313	325	326	327	328	329	330	332	333	334
WT	L	L	S	V	F	F	P	P	P	V	V	V	D	V	S	E	Y	N	S	T	W	N	K	A	L	P	A	I	E	K
153																			H											
154																				H										
155																											V			
156																											I			
157																											F			
158																											R			
159																											H			
160																						D								
161																						E								
162																						A								
163																						T								
164																						V								
165																						H								
166																									D		E			
167																									E		E			
168																									N		E			
169																									Q		E			
170																									V		E			
171																									T		E			
172																									H		E			
173																									I		E			
174																									A					
175																											T			
176																											H			
177																											Y			
178																											A			
179			E									I															E			
180			Q									I															E			
181			E									I															Y	E		
182			E									I							A								Y	E		

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Table 60 (continued)

Position	234	235	239	240	241	243	244	245	247	262	263	264	265	266	267	269	296	297	298	299	313	325	326	327	328	329	330	332	333	334
WT	L	L	S	V	F	F	P	P	P	V	V	V	D	V	S	E	Y	N	S	T	W	N	K	A	L	P	A	I	E	K
183			D															D										E		
184			E															D										E		
185			D										V					D										E		
186			D										I					D										E		
187			D										L					D										E		
188			D										F					D										E		
189			D										Y					D										E		
190			D										H					D										E		
191			D										T					D										E		
192												E						D										E		
193																	D	D										E		
194																	E	D										E		
195																	N	D										E		
196																	Q	D										E		
197																	H	D										E		
198																	T	D										E		
199																		D		V								E		
200																		D		I								E		
201																		D		L								E		
202																		D		F								E		
203																		D		H								E		
204																		D		E								E		
205																		D									Y	E		
206																		D	A								Y	E		
207			D																								Y	E		
208			N																								Y	E		
209			D																								L	E		
210			N																								L	E		
211												I							A									E		
212			D																A									E		

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Table 60 (continued)

Position	234	235	239	240	241	243	244	245	247	262	263	264	265	266	267	269	296	297	298	299	313	325	326	327	328	329	330	332	333	334
WT	L	L	S	V	F	F	P	P	P	V	V	V	D	V	S	E	Y	N	S	T	W	N	K	A	L	P	A	I	E	K
213			N																A									E		
214			D									I																E		
215			D									I							A									E		
216			D									I															L	E		

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[203] Example 2: Experimental production and screening of Fc libraries

The majority of experimentation on the Fc variants was carried out in the context of the anti-cancer antibody alemtuzumab (Campath®, a registered trademark of Ilex Pharmaceuticals LP). Alemtuzumab binds a short linear epitope within its target antigen CD52 (Hale *et al.*, 1990, *Tissue Antigens* 35:118-127; Hale, 1995, *Immunotechnology* 1:175-187). Alemtuzumab has been chosen as the primary engineering template because its efficacy is due in part to its ability to recruit effector cells (Dyer *et al.*, 1989, *Blood* 73:1431-1439; Friend *et al.*, 1991, *Transplant Proc* 23:2253-2254; Hale *et al.*, 1998, *Blood* 92:4581-4590; Glennie *et al.*, 2000, *Immunol Today* 21:403-410), and because production and use of its antigen in binding assays are relatively straightforward. In order to evaluate the optimized Fc variants of the present invention in the context of other antibodies, select Fc variants were evaluated in the anti-CD20 antibody rituximab (Rituxan®, a registered trademark of IDEC Pharmaceuticals Corporation), and the anti-Her2 antibody trastuzumab (Herceptin®, a registered trademark of Genentech). The use of alemtuzumab, rituximab, and trastuzumab for screening purposes is not meant to constrain the present invention to any particular antibody.

[204] The IgG1 full length light (V_L-C_L) and heavy (V_H-Cγ1-Cγ2-Cγ3) chain antibody genes for alemtuzumab, rituximab, and trastuzumab were constructed with convenient end restriction sites to facilitate subcloning. The genes were ligated into the mammalian expression vector pcDNA3.1Zeo (Invitrogen). The V_H-Cγ1-Cγ2-Cγ3 clone in pcDNA3.1zeo was used as a template for mutagenesis of the Fc region. Mutations were introduced into this clone using PCR-based mutagenesis techniques. Fc variants were sequenced to confirm the fidelity of the sequence. Plasmids containing heavy chain gene (V_H-Cγ1-Cγ2-Cγ3) (wild-type or variants) were co-transfected with plasmid containing light chain gene (V_L-C_L) into 293T cells. Media were harvested 5 days after transfection. Expression of immunoglobulin was monitored by screening the culture supernatant of transfectomas by western using peroxidase-conjugated goat-anti human IgG (Jackson ImmunoResearch, catalog # 109-035-088). Figure 6 shows expression of wild-type alemtuzumab and variants 1 through 10 in 293T cells. Antibodies were purified from the supernatant using protein A affinity chromatography (Pierce, Catalog # 20334. Figure 7 shows results of the protein purification for WT alemtuzumab. Antibody Fc variants showed similar expression and purification results to WT. Some Fc variants were deglycosylated in order to determine their solution and functional properties in the absence of carbohydrate. To obtain deglycosylated antibodies, purified alemtuzumab antibodies were incubated with peptide-N-glycosidase (PNGase F) at 37°C for 24h. Figure 8 presents an SDS PAGE gel confirming deglycosylation for several Fc variants and WT alemtuzumab.

[205] In order to confirm the functional fidelity of alemtuzumab produced under these conditions, the antigenic CD52 peptide, fused to GST, was expressed in E.coli BL21 (DE3) under IPTG induction. Both un-induced and induced samples were run on a SDS PAGE gel, and transferred to PVDF membrane. For western analysis, either alemtuzumab from Sotec (final concentration 2.5ng/ul) or

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media of transfected 293T cells (final alemtuzumab concentration about 0.1-0.2ng/ul) were used as primary antibody, and peroxidase-conjugated goat-anti human IgG was used as secondary antibody. Figure 9 presents these results. The ability to bind target antigen confirms the structural and functional fidelity of the expressed alemtuzumab. Fc variants that have the same variable region as WT alemtuzumab are anticipated to maintain a comparable binding affinity for antigen.

[206] In order to screen for Fc/FcγR binding, the extracellular regions of human V158 FcγRIIIa, human F158 FcγRIIIa, human FcγRIIb, human FcγRIIa, and mouse FcγRIII, were expressed and purified. Figure 10 presents an SDS PAGE gel that shows the results of expression and purification of human V158 FcγRIIIa. The extracellular region of this receptor was obtained by PCR from a clone obtained from the Mammalian Gene Collection (MGC:22630). The receptor was fused with glutathione S-Transferase (GST) to enable screening. Tagged Fc□RIIIa was transfected in 293T cells, and media containing secreted Fc□RIIIa were harvested 3 days later and purified. For western analysis, membrane was probed with anti-GST antibody.

[207] Binding affinity to Fc□RIIIa and FcγRIIb was measured for all designed Fc variants using an AlphaScreen™ assay (Amplified Luminescent Proximity Homogeneous Assay (ALPHA), PerkinElmer, Wellesley, MA), a bead-based non-radioactive luminescent proximity assay. Laser excitation of a donor bead excites oxygen, which if sufficiently close to the acceptor bead generates a cascade of chemiluminescent events, ultimately leading to fluorescence emission at 520-620 nm. The AlphaScreen™ assay was applied as a competition assay for screening Fc variants. WT alemtuzumab antibody was biotinylated by standard methods for attachment to streptavidin donor beads, and GST-tagged FcγR was bound to glutathione chelate acceptor beads. In the absence of competing Fc variants, WT antibody and FcγR interact and produce a signal at 520-620 nm. Addition of untagged Fc variant competes with the WT Fc/FcγR interaction, reducing fluorescence quantitatively to enable determination of relative binding affinities. All Fc variants were screened for V158 FcγRIIIa binding using the AlphaScreen™ assay. Select Fc variants were subsequently screened for binding to FcγRIIb, as well as other FcγRs and Fc ligands.

[208] Figure 11 shows AlphaScreen™ data for binding to human V158 FcγRIIIa by select Fc variants. The binding data were normalized to the maximum and minimum luminescence signal provided by the baselines at low and high concentrations of competitor antibody respectively. The data were fit to a one site competition model using nonlinear regression, and these fits are represented by the curves in the figure. These fits provide the inhibitory concentration 50% (IC50) (i.e. the concentration required for 50% inhibition) for each antibody, illustrated by the dotted lines in Figure 11, thus enabling the relative binding affinities of Fc variants to be quantitatively determined. Here, WT alemtuzumab has an IC50 of $(4.63 \times 10^{-9}) \times (2) = 9.2$ nM, whereas S239D has an IC50 of $(3.98 \times 10^{-10}) \times (2) = 0.8$ nM. Thus S239D alemtuzumab binds 9.2 nM / 0.8 nM = 11.64-fold more tightly

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than WT alemtuzumab to human V158 FcγRIIIa. Similar calculations were performed for the binding of all Fc variants to human V158 FcγRIIIa. Select Fc variants were also screened for binding to human Fc□RIIb, and examples of these AlphaScreen™ binding data are shown in Figure 12. Table 61 presents the fold-enhancement or fold-reduction relative to the parent antibody for binding of Fc variants to human V158 FcγRIIIa (column 3) and human FcγRIIb (column 4), as determined by the AlphaScreen™ assay. For these data, a fold above 1 indicates an enhancement in binding affinity, and a fold below 1 indicates a reduction in binding affinity relative to WT Fc. All data were obtained in the context of alemtuzumab, except for those indicated with an asterix (*), which were tested in the context of trastuzumab.

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Table 61

Variant	Substitution(s)	FcγRIIIa Fold	FcγRIIb Fold	FcγIIIa-fold : FcγIIb-fold
1	V264A	0.53		
2	V264L	0.56		
3	V264I	1.43		
4	F241W	0.29		
5	F241L	0.26		
6	F243W	0.51		
7	F243L	0.51		
8	F241L/F243L/V262I/V264I	0.09		
9	F241W/F243W	0.07		
10	F241W/F243W/V262A/V264A	0.04		
11	F241L/V262I	0.06		
12	F243L/V264I	1.23		
13	F243L/V262I/V264W	0.02		
14	F241Y/F243Y/V262T/V264T	0.05		
15	F241E/F243R/V262E/V264R	0.05		
16	F241E/F243Q/V262T/V264E	0.07		
17	F241R/F243Q/V262T/V264R	0.02		
18	F241E/F243Y/V262T/V264R	0.05		
19	L328M	0.21		
20	L328E	0.12		
21	L328F	0.24		
22	I332E	6.72	3.93	1.71
23	L328M/I332E	2.60		
24	P244H	0.83		
25	P245A	0.25		
26	P247V	0.53		
27	W313F	0.88		
28	P244H/P245A/P247V	0.93		
29	P247G	0.54		
30	V264I/I332E	12.49	1.57*	7.96
31	F241E/F243R/V262E/V264R/I332E	0.19		
32	F241E/F243Q/V262T/V264E/I332E			
33	F241R/F243Q/V262T/V264R/I332E			
34	F241E/F243Y/V262T/V264R/I332E	0.10		
35	S298A	2.21		
36	S298A/I332E	21.73		
37	S298A/E333A/K334A	2.56		
41	S239E/I332E	5.80	3.49	1.66
42	S239Q/I332E	6.60	4.68	1.41
43	S239E	10.16		
44	D265G	<0.02		
45	D265N	<0.02		
46	S239E/D265G	<0.02		
47	S239E/D265N	0.02		
48	S239E/D265Q	0.05		
49	Y296E	0.73	1.11	0.66
50	Y296Q	0.52	0.43	1.21

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Table 61 (continued)

Variant	Substitution(s)	FcγRIIIa Fold	FcγRIIb Fold	FcγIIIa-fold : FcγIIb-fold
51	S298T	0.94	<0.02	
52	S298N	0.41	<0.02	
53	T299I	<0.02		
54	A327S	0.23	0.39	0.59
55	A327N	0.19	1.15	0.17
56	S267Q/A327S	0.03		
57	S267L/A327S	<0.02		
58	A327L	0.05		
59	P329F	<0.02		
60	A330L	0.73	0.38	1.92
61	A330Y	1.64	0.75	2.19
62	I332D	17.80	3.34	5.33
63	N297S	<0.02		
64	N297D	<0.02		
65	N297S/I332E	<0.02		
66	N297D/I332E	0.08	<0.02	
67	N297E/I332E	<0.02		
68	D265Y/N297D/I332E	<0.02		
69	D265Y/N297D/T299L/I332E	<0.02		
70	D265F/N297E/I332E	<0.02		
71	L328I/I332E	7.03		
72	L328Q/I332E	1.54		
73	I332N	0.39		
74	I332Q	0.37		
75	V264T	2.73		
76	V264F	0.16		
77	V240I	3.25		
78	V263I	0.10		
79	V266I	1.86		
80	T299A	0.03		
81	T299S	0.15		
82	T299V	<0.02		
83	N325Q	<0.02		
84	N325L	<0.02		
85	N325I	<0.02		
86	S239D	11.64	4.47*	2.60
87	S239N	<0.02		
88	S239F	0.22	<0.02	
89	S239D/I332D	14.10		
90	S239D/I332E	56.10	19.71*	2.85
91	S239D/I332N	7.19		
92	S239D/I332Q	9.28		
93	S239E/I332D	9.33		
94	S239E/I332N	11.93		
95	S239E/I332Q	3.80		
96	S239N/I332D	3.08		
97	S239N/I332E	14.21		
98	S239N/I332N	0.43		

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Table 61 (continued)

Variant	Substitution(s)	FcγRIIIa Fold	FcγRIIb Fold	FcγIIIa-fold : FcγIIb-fold
99	S239N/I332Q	0.56		
100	S239Q/I332D	5.05		
101	S239Q/I332N	0.39		
102	S239Q/I332Q	0.59		
103	K326E	3.85		
104	Y296D	0.62		
105	Y296N	0.29		
106	F241Y/F243Y/V262T/V264T/N297D/I332E	0.15		
107	A330Y/I332E	12.02	4.40	2.73
108	V264I/A330Y/I332E	12.00	3.54	3.39
109	A330L/I332E	10.34	2.03	5.09
110	V264I/A330L/I332E	11.15	1.79	6.23
111	L234D	0.21		
112	L234E	1.34	2.21	0.61
113	L234N	0.56	1.39	0.40
114	L234Q	0.37		
115	L234T	0.35		
116	L234H	0.33		
117	L234Y	1.42	1.08	1.31
118	L234I	1.55	1.14	1.36
119	L234V	0.38		
120	L234F	0.30		
121	L235D	1.66	3.63	0.46
122	L235S	1.25		
123	L235N	0.40		
124	L235Q	0.51		
125	L235T	0.52		
126	L235H	0.41		
127	L235Y	1.19	10.15	0.12
128	L235I	1.10	0.94	1.17
129	L235V	0.48		
130	L235F	0.73	3.53	0.21
131	S239T	1.34		
132	S239H	0.20		
133	S239Y	0.21		
134	V240A	0.70	0.14	5.00
135	V240T			
136	V240M	2.06	1.38	1.49
137	V263A			
138	V263T	0.43		
139	V263M	0.05		
140	V264M	0.26		
141	V264Y	1.02	0.27	3.78
142	V266A	<0.02		
143	V266T	0.45		
144	V266M	0.62		
145	E269H	<0.02		
146	E269Y	0.12		

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Table 61 (continued)

Variant	Substitution(s)	FcγRIIIa Fold	FcγRIIb Fold	FcγIIIa-fold : FcγIIb-fold
147	E269F	0.16		
148	E269R	0.05		
149	Y296S	0.12		
150	Y296T	<0.02		
151	Y296L	0.22		
152	Y296I	0.09		
153	A298H	0.27		
154	T299H	<0.02		
155	A330V	0.43		
156	A330I	1.71	0.02	85.5
157	A330F	0.60		
158	A330R	<0.02		
159	A330H	0.52		
160	N325D	0.41		
161	N325E	<0.02		
162	N325A	0.11		
163	N325T	1.10		
164	N325V	0.48		
165	N325H	0.73		
166	L328D/I332E	1.34		
167	L328E/I332E	0.20		
168	L328N/I332E	<0.02		
169	L328Q/I332E	0.70		
170	L328V/I332E	2.06		
171	L328T/I332E	1.10		
172	L328H/I332E	<0.02		
173	L328I/I332E	3.49		
174	L328A	0.20		
175	I332T	0.72		
176	I332H	0.46		
177	I332Y	0.76		
178	I332A	0.89		
179	S239E/V264I/I332E	15.46		
180	S239Q/V264I/I332E	2.14		
181	S239E/V264I/A330Y/I332E	8.53		
182	S239E/V264I/S298A/A330Y/I332E			
183	S239D/N297D/I332E	0.28		
184	S239E/N297D/I332E	0.06		
185	S239D/D265V/N297D/I332E			
186	S239D/D265I/N297D/I332E			
187	S239D/D265L/N297D/I332E	<0.02		
188	S239D/D265F/N297D/I332E	<0.02		
189	S239D/D265Y/N297D/I332E	0.02		
190	S239D/D265H/N297D/I332E	0.04		
191	S239D/D265T/N297D/I332E	<0.02		
192	V264I/N297D/I332E	0.05		
193	Y296D/N297D/I332E			
194	Y296E/N297D/I332E	<0.02		

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Table 61 (continued)

Variant	Substitution(s)	FcγRIIIa Fold	FcγRIIb Fold	FcγIIIa-fold : FcγIIb-fold
195	Y296N/N297D/I332E	0.04		
196	Y296Q/N297D/I332E	<0.02		
197	Y296H/N297D/I332E	<0.02		
198	Y296T/N297D/I332E	<0.02		
199	N297D/T299V/I332E	<0.02		
200	N297D/T299I/I332E	<0.02		
201	N297D/T299L/I332E	<0.02		
202	N297D/T299F/I332E	<0.02		
203	N297D/T299H/I332E	<0.02		
204	N297D/T299E/I332E	<0.02		
205	N297D/A330Y/I332E	0.43		
206	N297D/S298A/A330Y/I332E			
207*	S239D/A330Y/I332E	129.58		
208*	S239N/A330Y/I332E	14.22		
209*	S239D/A330L/I332E	138.63	7.50	18.48
210*	S239N/A330L/I332E	12.95		
211*	V264I/S298A/I332E	16.50		
212*	S239D/S298A/I332E	295.16	6.16	47.92
213*	S239N/S298A/I332E	32.14	5.15	6.24
214*	S239D/V264I/I332E	36.58	14.39	2.54
215*	S239D/V264I/S298A/I332E			
216*	S239D/V264I/A330L/I332E			

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[209] Example 3: Selectively enhanced binding to FcγRs

A number of promising Fc variants with optimized properties were obtained from the FcγRIIIa and FcγRIIb screen. Table 61 provides Fc variants that bind more tightly to FcγRIIIa, and thus are candidates for improving the effector function of antibodies and Fc fusions. These include a number of variants that comprise substitutions at 239, 264, 330, and 332. Figure 13 shows AlphaScreen™ binding data for some of these Fc variants. The majority of these Fc variants provide substantially greater FcγRIIIa binding enhancements over S298A/E333A/K334A.

[210] Although the majority of Fc variants were screened in the context of the antibody alemtuzumab, select Fc variants were also screened in the context of rituximab and trastuzumab. AlphaScreen™ data for binding of select Fc variants to human V158 FcγRIIIa in the context of rituximab and trastuzumab are shown in Figures 14 and 15 respectively. The results indicate that the Fc variants display consistent binding enhancements regardless of the antibody context, and thus the Fc variants of the present invention are broadly applicable to antibodies and Fc fusions.

[211] Fc variants have been obtained that show differentially enhanced binding to FcγRIIIa over FcγRIIb. As discussed, optimal effector function may result from Fc variants wherein affinity for activating FcγRs is greater than affinity for the inhibitory FcγRIIb. AlphaScreen™ data directly comparing binding to FcγRIIIa and FcγRIIb for two Fc variants with this specificity profile are shown in Figures 16a and 16b. This concept can be defined quantitatively as the fold-enhancement or -reduction of the activating FγR (Table 61, column 3) divided by the fold-enhancement or -reduction of the inhibitory FcγR (Table 61, column 4), herein referred to as the FcγRIIIa-fold:FcγRIIb-fold ratio. This value provided in Column 5 in Table 61. Table 61 shows that Fc variants provide this specificity profile, with a FcγRIIIa-fold:FcγRIIb-fold ratio as high as 86:1.

[212] Some of the most promising Fc variants of the present invention for enhancing effector function have both substantial increases in affinity for FcγRIIIa and favorable FcγRIIIa-fold:FcγRIIb-fold ratios. These include, for example, S239D/I332E (FcγRIIIa-fold = 56, FcγRIIIa-fold:FcγRIIb-fold = 3), S239D/A330Y/I332E (FcγRIIIa-fold = 130), S239D/A330L/I332E (FcγRIIIa-fold = 139, FcγRIIIa-fold:FcγRIIb-fold = 18), and S239D/S298A/I332E (FcγRIIIa-fold = 295, FcγRIIIa-fold:FcγRIIb-fold = 48). Figure 17 shows AlphaScreen™ binding data for these and other Fc variants to human V158 FcγRIIIa.

[213] Because there are a number of FcγRs that contribute to effector function, it may be worthwhile to additionally screen Fc variants against other receptors. Figure 18 shows AlphaScreen™ data for binding of select Fc variants to human R131 FcγRIIa. As can be seen, those aforementioned variants

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with favorable binding enhancements and specificity profiles also show enhanced binding to this activating receptor. The use of FcγRIIIa, FcγRIIb, and FcγRIIc for screening is not meant to constrain experimental testing to these particular FcγRs; other FcγRs are contemplated for screening, including but not limited to the myriad isoforms and allotypes of FcγRI, FcγRII, and FcγRIII from humans, mice, rats, monkeys, and the like, as previously described.

[214] Taken together, the FcγR binding data provided in Figures 11 – 18 and Table 61 indicate that a number of substitions at positions 234, 235, 239, 240, 243, 264, 266, 325, 328, 330, and 332 are promising candidates for improving the effector function of antibodies and Fc fusions. Because combinations of some of these substitutions have typically resulted in additive or synergistic binding improvements, it is anticipated that as yet unexplored combinations of the Fc variants provided in Table 61 will also provide favorable results. Thus all combinations of the Fc variants in Table 61 are contemplated. Likewise, combinations of any of the Fc variants in Table 61 with other discovered or undiscovered Fc variants may also provide favorable properties, and these combinations are also contemplated. Furthermore, it is anticipated from these results that other substitutions at positions 234, 235, 239, 240, 243, 264, 266, 325, 328, 330, and 332 may also provide favorable binding enhancements and specificities, and thus substitutions at these positions other than those presented in Table 61 are contemplated.

[215] Example 4: Reduced binding to FcγRs

As discussed, although there is a need for greater effector function, for some antibody therapeutics, reduced or eliminated effector function may be desired. Several Fc variants in Table 61 substantially reduce or ablate FcγR binding, and thus may find use in antibodies and Fc fusions wherein effector function is undesirable. AlphaScreen™ binding data for some examples of such variants are shown in Figures 19a and 19b. These Fc variants, as well as their use in combination, may find use for eliminating effector function when desired, for example in antibodies and Fc fusions whose mechanism of action involves blocking or antagonism but not killing of the cells bearing target antigen.

[216] Example 5: Aglycosylated Fc variants

As discussed, one goal of the current experiments was to obtain optimized aglycosylated Fc variants. Several Fc variants provide significant progress towards this goal. Because it is the site of glycosylation, substitution at N297 results in an aglycosylated Fc. Whereas all other Fc variants that comprise a substitution at N297 completely ablate FcγR binding, N297D/I332E has significant binding affinity for FcγRIIIa, shown in Table 61 and illustrated in Figure 20. The exact reason for this result is uncertain in the absence of a high-resolution structure for this variant, although the computational screening predictions suggest that it is potentially due to a combination of new favorable Fc/FcγR interactions and favorable electrostatic properties. Indeed other electrostatic substitutions are

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envisioned for further optimization of aglycosylated Fc. Table 61 shows that other aglycosylated Fc variants such as S239D/N297D/I332E and N297D/A330Y/I332E provide binding enhancements that bring affinity for FcγRIIIa within 0.28- and 0.43-fold respectively of glycosylated WT alemtuzumab. Combinations of these variants with other Fc variants that enhance FcγR binding are contemplated, with the goal of obtaining aglycosylated Fc variants that bind one or more FcγRs with affinity that is approximately the same as or even better than glycosylated parent Fc. An additional set of promising Fc variants provide stability and solubility enhancements in the absence of carbohydrate. Fc variants that comprise substitutions at positions 241, 243, 262, and 264, positions that do not mediate FγR binding but do determine the interface between the carbohydrate and Fc, ablate FγR binding, presumably because they perturb the conformation of the carbohydrate. In deglycosylated form, however, Fc variants F241E/F243R/V262E/V264R, F241E/F243Q/V262T/V264E, F241R/F243Q/V262T/V264R, and F241E/F243Y/V262T/V264R show stronger binding to FcγRIIIa than in glycosylated form, as shown by the AlphaScreen™ data in Figure 21. This result indicates that these are key positions for optimization of the structure, stability, solubility, and function of aglycosylated Fc. Together these results suggests that protein engineering can be used to restore the favorable functional and solution properties of antibodies and Fc fusions in the absence of carbohydrate, and pave the way for aglycosylated antibodies and Fc fusions with favorable solution properties and full functionality that comprise substitutions at these and other Fc positions.

[217] Example 6. Affinity of Fc variants for polymorphic forms of FcγRIIIa

As discussed above, an important parameter of Fc-mediated effector function is the affinity of Fc for both V158 and F158 polymorphic forms of FcγRIIIa. AlphaScreen™ data comparing binding of select variants to the two receptor allotypes are shown in Figure 22a (V158 FcγRIIIa) and Figure 22b (F158 FcγRIIIa). As can be seen, all variants improve binding to both FcγRIIIa allotypes. These data indicate that those Fc variants of the present invention with enhanced effector function will be broadly applicable to the entire patient population, and that enhancement to clinical efficacy will potentially be greatest for the low responsive patient population who need it most.

[218] The FcγR binding affinities of these Fc variants were further investigated using Surface Plasmon Resonance (SPR) (Biacore, Uppsala, Sweden). SPR is a sensitive and extremely quantitative method that allows for the measurement of binding affinities of protein-protein interactions, and has been used to effectively measure Fc/FcγR binding (Radaev *et al.*, 2001, *J Biol Chem* 276:16478-16483). SPR thus provides an excellent complementary binding assay to the AlphaScreen™ assay. His-tagged V158 FcγRIIIa was immobilized to an SPR chip, and WT and Fc variant alemtuzumab antibodies were flowed over the chip at a range of concentrations. Binding constants were obtained from fitting the data using standard curve-fitting methods. Table 62 presents dissociation constants (Kd) for binding of select Fc variants to V158 FcγRIIIa and F158 FcγRIIIa obtained using SPR, and compares these with IC50s obtained from the AlphaScreen™ assay. By

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dividing the Kd and IC50 for each variant by that of WT alemtuzumab, the fold-improvements over WT (Fold) are obtained.

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Table 62

	SPR V158 FcγRIIIa		SPR F158 FcγRIIIa		AlphaScreen™ V158 FcγRIIIa		AlphaScreen™ F158 FcγRIIIa	
	Kd (nM)	Fold	Kd (nM)	Fold	IC50 (nM)	Fold	IC50 (nM)	Fold
WT	68		730		6.4		17.2	
V264I	64	1.1	550	1.3	4.5	1.4	11.5	1.5
I332E	31	2.2	72	10.1	1.0	6.4	2.5	6.9
V264I/I332E	17	4.0	52	14.0	0.5	12.8	1.1	15.6
S298A	52	1.3	285	2.6	2.9	2.2	12.0	1.4
S298A/E333A/ K334A	39	1.7	156	4.7	2.5	2.6	7.5	2.3

[219] The SPR data corroborate the improvements to FcγRIIIa affinity observed by AlphaScreen™ assay. Table 62 further indicates the superiority of V264I/I332E and I332E over S298A and S298A/E333A/K334A; whereas S298A/E333A/K334A improves Fc binding to V158 and F158 FcγRIIIa by 1.7-fold and 4.7-fold respectively, I332E shows binding enhancements of 2.2-fold and 10.1-fold respectively, and V264I/I332E shows binding enhancements of 4.0-fold and 14-fold respectively. Also worth noting is that the affinity of V264I/I332E for F158 FcγRIIIa (52 nM) is better than that of WT for the V158 allotype (68 nM), suggesting that this Fc variant, as well as those with even greater improvements in binding, may enable the clinical efficacy of antibodies for the low responsive patient population to achieve that currently possible for high responders. The correlation between the SPR and AlphaScreen™ binding measurements are shown in Figures 23a – 23d. Figures 23a and 23b show the Kd - IC50 correlations for binding to V158 FcγRIIIa and F158 FcγRIIIa respectively, and Figures 23c and 23d show the fold-improvement correlations for binding to V158 FcγRIIIa and F158 FcγRIIIa respectively. The good fits of these data to straight lines (r^2 = 0.9, r^2 = 0.84, r^2 = 0.98, and r^2 = 0.90) support the accuracy the AlphaScreen™ measurements, and validate its use for determining the relative Fc□R binding affinities of Fc variants.

[220] Example 7. ADCC of Fc variants

In order to determine the effect on effector function, cell-based ADCC assays were performed on select Fc variants. ADCC was measured using the DELFIA® EuTDA-based cytotoxicity assay (Perkin Elmer, MA) with purified human peripheral blood monocytes (PBMCs) as effector cells. Target cells were loaded with BATDA at $1x10^6$ cells/ml, washed 4 times and seeded into 96-well plate at 10,000 cells/well. The target cells were then opsonized using Fc variant or WT antibodies at the indicated final concentration. Human PBMCs were added at the indicated fold-excess of target cells and the plate was incubated at 37°C for 4 hrs. The co-cultured cells were centrifuged at 500xg, supernatants were transferred to a separate plate and incubated with Eu solution, and relative fluorescence units were measured using a Packard Fusion™ reader (Packard Biosciences, IL). Samples were run in triplicate to provide error estimates (n=3, +/- S.D.). PBMCs were allotyped for the V158 or F158 FcγRIIIa allotype using PCR.

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[221] ADCC assays were run on Fc variant and WT alemtuzumab using DoHH-2 lymphoma target cells. Figure 24a is a bar graph showing the ADCC of these proteins at 10 ng/ml antibody. Results show that alemtuzumab Fc variants I332E, V264I, and I332E/V264I have substantially enhanced ADCC compared to WT alemtuzumab, with the relative ADCC enhancements proportional to their binding improvements to FcγRIIIa as indicated by AlphaScreen™ assay and SPR. The dose dependence of ADCC on antibody concentration is shown in Figure 24b. These data were normalized to the minimum and maximum fluorescence signal provided by the baselines at low and high concentrations of antibody respectively. The data were fit to a sigmoidal dose-response model using nonlinear regression, represented by the curve in the figure. The fits enable determination of the effective concentration 50% (EC50) (i.e. the concentration required for 50% effectiveness), which provides the relative enhancements to ADCC for each Fc variant. The EC50s for these binding data are analogous to the IC50s obtained from the AlphaScreen™ competition data, and derivation of these values is thus analogous to that described in Example 2 and Figure 11. In Figure 24b, the log(EC50)s, obtained from the fits to the data, for WT, V264I/I332E, and S239D/I332E alemtuzumab are 0.99, 0.60, and 0.49 respectively, and therefore their respective EC50s are 9.9, 4.0, and 3.0. Thus V264I/I332E and S239E/I332E provide a 2.5-fold and 3.3-fold enhancement respectively in ADCC over WT alemtuzumab using PBMCs expressing heterozygous V158/F158 FcγRIIIa. These data are summarized in Table 63 below.

Table 63

	log(EC50)	EC50 (ng/ml)	Fold Improvement Over WT
WT	0.99	9.9	
V264I/I332E	0.60	4.0	2.5
S239D/I332E	0.49	3.0	3.3

[222] In order to determine whether these ADCC enhancements are broadly applicable to antibodies, select Fc variants were evaluated in the context of rituximab and trastuzumab. ADCC assays were run on V264I/I332E, WT, and S298A/D333A/K334A rituximab using WIL2-S lymphoma target cells. Figure 25a presents a bar graph showing the ADCC of these proteins at 1 ng/ml antibody. Results indicate that V264I/I332E rituximab provides substantially enhanced ADCC relative to WT rituximab, as well as superior ADCC to S298A/D333A/K334A, consistent with the FcγRIIIa binding improvements observed by AlphaScreen™ assay and SPR. Figure 25b shows the dose dependence of ADCC on antibody concentration. The EC50s obtained from the fits of these data and the relative fold-improvements in ADCC are provided in Table 64 below. As can be seen V264I/I332E rituximab provides an 11.3-fold enhancement in EC50 over WT for PBMCs expressing homozygous F158/F158 FcγRIIIa. The greater improvements observed for rituximab versus alemtuzumab are

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likely due to the use of homozygous F158/F158 FcγRIIIa rather than heterozygous V158/F158 FcγRIIIa PBMCs, as well as potentially the use of different antibodies and target cell lines.

Table 64

	log(EC50)	EC50 (ng/ml)	Fold Improvement Over WT
WT	0.23	1.7	
S298A/E333A/K334A	-0.44	0.37	4.6
V264I/I332E	-0.83	0.15	11.3

[223] ADCC assays were run on Fc variant and WT trastuzumab using two breast carcinoma target cell lines BT474 and Sk-Br-3. Figure 26a shows a bar graph illustrating ADCC at 1 ng/ml antibody. Results indicate that V264I and V264I/I332E trastuzumab provide substantially enhanced ADCC compared to WT trastuzumab, with the relative ADCC enhancements proportional to their binding improvements to FcγRIIIa as indicated by AlphaScreen™ assay and SPR. Figure 26b shows the dose dependence of ADCC on antibody concentration. The EC50s obtained from the fits of these data and the relative fold-improvements in ADCC are provided in Table 65 below. Significant ADCC improvements are observed for I332E trastuzumab when combined with A330L and A330Y.

Table 65

	log(EC50)	EC50 (ng/ml)	Fold Improvement Over WT
WT	1.1	11.5	
I332E	0.34	2.2	5.2
A330Y/I332E	-0.04	0.9	12.8
A330L/I332E	0.04	1.1	10.5

[224] Figure 26c shows another set of dose response ADCC data at variable antibody concentrations for trastuzumab variants. The EC50s obtained from the fits of these data and the relative fold-improvements in ADCC are provided in Table 66 below. Results show that trastuzumab Fc variants S239D/I332E, S239D/S298A/I332E, S239D/A330Y/I332E, and S239D/A330L/I332E/ provide substantial ADCC enhancements relative to WT trastuzumab and S298A/E333A/K334A; consistent with the FcγR binding data observed by the AlphaScreen™ assay and SPR. S239D/A330L/I332E trastuzumab shows the largest increase in effector function observed thus far, providing an approximate 50-fold enhancement in EC50 over WT for PBMCs expressing homozygous F158/F158 FcγRIIIa.

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Table 66

	log(EC50)	EC50 (ng/ml)	Fold Improvement Over WT
WT	0.45	2.83	
S298A/E333A/K334A	-0.17	0.67	4.2
S239D/I332E	-0.18	0.66	4.3
S239D/A330Y/I332E	-0.29	0.51	5.5
S239D/S298A/I332E	-0.52	0.30	9.4
S239D/A330L/I332E	-1.22	0.06	47.2

[225] Example 8. Complement binding and activation by Fc variants

Complement protein C1q binds to a site on Fc that is proximal to the FcγR binding site, and therefore it was prudent to determine whether the Fc variants have maintained their capacity to recruit and activate complement. The AlphaScreen™ assay was used to measure binding of select Fc variants to the complement protein C1q. The assay was carried out with biotinylated WT alemtuzumab antibody attached to streptavidin donor beads as described in Example 2, and using C1q coupled directly to acceptor beads. Binding data of select Fc variants shown in Figure 27a indicate that C1q binding is uncompromised. Cell-based CDC assays were also performed on select Fc variants to investigate whether Fc variants maintain the capacity to activate complement. Amar Blue was used to monitor lysis of Fc variant and WT rituximab-opsonized WIL2-S lymphoma cells by human serum complement (Quidel, San Diego, CA). The results shown in Figure 27b for select Fc variants indicate that CDC is uncompromised.

[226] Example 9. Protein A binding by Fc variants

As discussed, bacterial protein A binds to the Fc region between the C□2 and C□3 domains, and is frequently employed for antibody purification. The AlphaScreen™ assay was used to measure binding of select Fc variants to the protein A using biotinylated WT alemtuzumab antibody attached to streptavidin donor beads as described in Example 2, and using protein A coupled directly to acceptor beads. The binding data shown in Figure 28 for select Fc variants indicate that the capacity of the Fc variants to bind protein A is uncompromised. These results suggest that affinity of the Fc variants for other Fc ligands that bind the same site on Fc as protein A, such as the neonatal Fc receptor FcRn and protein G, are also unaffected.

[227] Example 10. Capacity of Fc variants to bind mouse Fc□Rs

Optimization of Fc to nonhuman Fc□Rs may be useful for experimentally testing Fc variants in animal models. For example, when tested in mice (for example nude mice, SCID mice, xenograft mice, and/or transgenic mice), antibodies and Fc fusions that comprise Fc variants that are optimized for

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one or more mouse Fc□Rs may provide valuable information with regard to efficacy, mechanism of action, and the like. In order to evaluate whether the Fc variants of the present invention may be useful in such experiments, affinity of select Fc variants for mouse FcγRIII was measured using the AlphaScreen™ assay. The AlphaScreen™ assay was carried out using biotinylated WT alemtuzumab attached to streptavidin donor beads as described in Example 2, and GST-tagged mouse FcγRIII bound to glutathione chelate acceptor beads, expressed and purified as described in Example 2. These binding data are shown in Figure 29. Results show that some Fc variants that enhance binding to human FcγRIIIa also enhance binding to mouse FcγRIII. This result indicates that the Fc variants of the present invention, or other Fc variants that are optimized for nonhuman FcγRs, may find use in experiments that use animal models.

[228] Example 11. Validation of Fc variants expressed in CHO cells

Whereas the Fc variants of the present invention were expressed in 293T cells for screening purposes, large scale production of antibodies is typically carried out by expression in Chinese Hamster Ovary (CHO) cell lines. In order to evaluate the properties of CHO-expressed Fc variants, select Fc variants and WT alemtuzumab were expressed in CHO cells and purified as described in Example 2. Figure 30 shows AlphaScreen™ data comparing binding of CHO- and 293T- expressed Fc variant and WT alemtuzumab to human V158 FcγRIIIa. The results indicate that the Fc variants of the present invention show comparable FcγR binding enhancements whether expressed in 293T or CHO.

[229] Example 12. Therapeutic application of Fc variants

A number of Fc variants described in the present invention have significant potential for improving the therapeutic efficacy of anticancer antibodies. For illustration purposes, a number of Fc variants of the present invention have been incorporated into the sequence of the antibody rituximab. The WT rituximab light chain and heavy chain, described in US 5,736,137, are provided in Figures 31a and 32b. The improved anti-CD20 antibody sequences are provided in Figure 31c. The improved anti-CD20 antibody sequences comprise at least non-WT amino acid selected from the group consisting of X_1, X_2, X_3, X_4, X_5, and X_6. These improved anti-CD20 antibody sequences may also comprise a substitution Z_1. The use of rituximab here is solely an example, and is not meant to constrain application of the Fc variants to this antibody or any other particular antibody or Fc fusion.

[230] All references are herein expressly incorporated by reference.

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[231] Whereas particular embodiments of the invention have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous variations of the details may be made without departing from the invention as described in the appended claims.

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CLAIMS

We claim:

1. An antibody comprising an Fc variant portion comprising at least one amino acid modification in the Fc region of said parent Fc polypeptide, wherein said Fc variant modulates binding to an FcγR as compared to the parent Fc polypeptide.

2. An antibody according to claim 1 wherein said modulation is an increase in affinity of said antibody to said FcγR.

3. An antibody according to claim 1 wherein said Fc variant portion comprises at least one substitution at a position corresponding to a position of the human sequence selected from the group consisting of: 234, 235, 239, 240, 241, 243, 244, 245, 247, 262, 263, 264, 265, 266, 267, 269, 296, 297, 298, 299, 313, 325, 326, 327, 328, 329, 330, 332, 333, and 334.

4. An antibody according to claim 1, wherein said Fc variant comprises at least one substitution at a position corresponding to a position of the human sequence selected from the group consisting of: 240, 244, 245, 247, 262, 263, 266, 299, 313, 325, 328, and 332.

5. An antibody according to claim 1, wherein said Fc variant comprises a substitution at position 332.

6. An antibody according to claim 1, wherein at least one substitution is at a position selected from the group consisting of: 239, 264, 297, and 330, with the proviso that if said sequence is substantially human, said substitution is not S239A, V264A, N297A, N297Q, A330D, A330Q, A330K, or A330S.

7. An antibody according to claim 1, wherein said Fc variant comprises at least one substitution selected from the group consisting of 234D, 234E, 234N, 234Q, 234T, 234H, 234Y, 234I, 234V, 234F, 235D, 235S, 235N, 235Q, 235T, 235H, 235Y, 235I, 235V, 235F, 239D, 239E, 239N, 239Q, 239F, 239T, 239H, 239Y, 240I, 240A, 240T, 240M, 241W, 241L, 241Y, 241E, 241R, 243W, 243L 243Y, 243R, 243Q, 244H, 245A, 247V, 247G, 262I, 262A, 262T, 262E, 263I, 263A, 263T, 263M, 264L, 264I, 264W, 264T, 264R, 264F, 264M, 264Y, 264E, 265G, 265N, 265Q, 265Y, 265F, 265V, 265I, 265L, 265H, 265T, 266I, 266A, 266T, 266M, 267Q, 267L, 269H, 269Y, 269F, 269R, 296E, 296Q, 296D, 296N, 296S, 296T, 296L, 296I, 296H,

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297S, 297D, 297E, 298H, 299I, 299L, 299A, 299S, 299V, 299H, 299F, 299E, 313F, 325Q, 325L, 325I, 325D, 325E, 325A, 325T, 325V, 325H, 327N, 327L, 328M, 328D, 328E, 328N, 328Q, 328F, 328I, 328V, 328T, 328H, 328A, 329F, 330L, 330Y, 330V, 330I, 330F, 330R, 330H, 332D, 332E, 332N, 332Q, 332T, 332H, 332Y, and 332A.

8. An antibody according to claim 1, wherein said Fc variant is selected from the group consisting of 264L, 264I, 241W, 241L, 243W, 243L, 241L/243L/262I/264I, 241W/243W, 241W/243W/262A/264A, 241L/262I, 243L/264I, 243L/262I/264W, 241Y/243Y/262T/264T, 241E/243R/262E/264R, 241E/243Q/262T/264E, 241R/243Q/262T/264R, 241E/243Y/262T/264R, 328M, 328E, 328F, 332E, 328M/332E, 244H, 245A, 247V, 313F, 244H/245A/247V, 247G, 264I/332E, 241E/243R/262E/264R/332E, 241E/243Q/262T/264E/332E, 241R/243Q/262T/264R/332E, 241E/243Y/262T/264R/332E, 298A/332E, 239EI332E, 239Q/332E, 239E, 265G, 265N, 239E/265G, 239E/265N, 239E/265Q, 296E, 296Q, 299I, 327N, 267Q/327S, 267L/327S , 327L, 329F, 330L, 330Y, 332D, 297S, 297D, 297S/332E, 297D/332E, 297E/332E, 265Y/297D/332E, 265Y/297D/299L/332E, 265F/297E/332E, 328I/332E, 328Q/332E, 332N, 332Q, 264T, 264F, 240I, 263I, 266I, 299A, 299S, 299V, 325Q, 325L, 325I, 239D, 239N, 239F, 239D/332D, 239D/332E, 239D/332N, 239D/332Q, 239E/332D, 239E/332N, 239E/332Q, 239N/332D, 239N/332E, 239N/332N, 239NI332Q, 239Q/332D, 239Q/332N, 239Q/332Q, 296D, 296N, 241Y/243Y/262T/264T/297D/332E, 330Y/332E, 264I/330Y/332E, 330L/332E, 264I/330L/332E, 234D, 234E, 234N, 234Q, 234T, 234H, 234Y, 234I, 234V, 234F, 235D, 235S, 235N, 235Q, 235T, 235H, 235Y, 235I, 235V, 235F, 239T, 239H, 239Y, 240A, 240T, 240M, 263A, 263T, 263M, 264M, 264Y, 266A, 266T, 266M, 269H, 269Y, 269F, 269R, 296S, 296T, 296L, 296I, 298H, 299H, 330V, 330I, 330F, 330R, 330H, 325D, 325E, 325A, 325T, 325V, 325H, 328D/332E, 328E/332E, 328N/332E, 328Q/332E, 328V/332E, 328T/332E, 328H/332E, 328I/332E, 328A, 332T, 332H, 332Y, 332A, 239E/264I/332E, 239Q/264I/332E, 239E/264I/330Y/332E, 239E/264I/298A/330Y/332E, 239D/297D/332E, 239E/297D/332E, 239D/265V/297D/332E, 239D/265I/297D/332E, 239D/265L/297D/332E, 239D/265F/297D/332E, 239D/265Y/297D/332E, 239D/265H/297D/332E, 239D/265T/297D/332E, 264E/297D/332E, 296D/297D/332E, 296E/297D/332E, 296N/297D/332E, 296Q/297D/332E, 296H/297D/332E, 296T/297D/332E, 297D/299V/332E, 297D/299I/332E, 297D/299L/332E, 297D/299F/332E, 297D/299H/332E, 297D/299E/332E, 297D/330Y/332E, 297D/298A/330YI332E, 239D/330Y/332E, 239N/330Y/332E, 239D/330L/332E, 239N/330L/332E, 264I/298A/332E, 239D/298A/332E, 239N/298A/332E, 239D/264I/332E, 239D/264I/298A/332E, and 239D/264I/330L/332E.

9. An antibody according to claim 7, wherein said Fc variant portion further comprises at least one substitution at a position corresponding to a position of the human sequence selected from the group consisting of: 256, 270, 290, 298, 312, 322, 326, 329, 331, 333, 334, and 339.

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10. An antibody according to claim 1, wherein said FcγR is FcγRIIIa.

11. An antibody according to claim 10, wherein said FcγRIIIa is a V158 or F158 allotype of FcγRIIIa.

12. An Fc variant according to claim 2, wherein said parent Fc polypeptide is substantially human, and said affinity is approximately 5-fold greater than that of said parent Fc polypeptide.

13. An antibody according to claim 12, wherein said parent Fc polypeptide is substantially human, and said affinity is between about 5-fold and about 300-fold greater than that of said parent Fc polypeptide.

14. An antibody according to claim 12, wherein said Fc variant portion comprises at least one substitution at a position corresponding to a position of the human sequence selected from the group consisting 234, 235, 239, 240, 243, 264, 266, 328, 330, 332, and 325.

15. An antibody according to claim 14, wherein said Fc variant comprises at least one substitution selected from the group consisting of 234E, 234Y, 234I, 235D, 235S, 235Y, 235I, 239D, 239E, 239N, 239Q, 239T, 240I, 240M, 243L, 264I, 264T, 264Y, 266I, 328M, 328I, 328Q, 328D, 328V, 328T, 330Y, 330L, 330I, 332D, 332E, 332N, 332Q, and 325T.

16. An antibody according to claim 15, wherein said Fc variant is selected from the group consisting of 264I, 243L/264I, 328M, 332E, 328M/332E, 264I/332E, 298A/332E, 239E/332E, 239Q/332E, 239E, 330Y, 332D, 328I/332E, 328Q/332E, 264T, 240I, 266I, 239D, 239D/332D, 239D/332E, 239D/332N, 239D/332Q, 239E/332D, 239E/332N, 239E/332Q, 239N/332D, 239N/332E, 239Q/332D, 330Y/332E, 264I/330Y/332E, 330L/332E, 264I/330L/332E, 234E, 234Y, 234I, 235D, 235S, 235Y, 235I, 239T, 240M, 264Y, 330I, 325T, 328D/332E, 328V/332E, 328T/332E, 328I/332E, 239E/264I/332E, 239Q/264I/332E, 239E/264I/330Y/332E, 239D/330Y/332E, 239N/330Y/332E, 239D/330L/332E, 239N/330L/332E, 264I/298A/332E, 239D/298A/332E, 239N/298A/332E, 239D/264I/332E, 239D/264I/298A/332E, and 239D/264I/330L/332E.

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17. An antibody according to claim 15, wherein said Fc variant further comprises at least one substitution at a position selected from the group consisting of 256, 270, 290, 298, 312, 322, 326, 329, 331, 333, 334, and 339.

18. An antibody according to claim 1 or 2, wherein said parent Fc polypeptide is substantially human, substantially mouse, substantially rat, or substantially monkey.

19. An antibody according to claim 1 or 2, wherein binding to one or more Fc ligands is unaltered.

20. An antibody according to claim 19, wherein said Fc ligand is selected from the group consisting of C1q, FcRn, protein A, and protein G.

21. An antibody according to claim 1 or 2, wherein CDC is unaffected.

22. An antibody according to claim 1 or 2, wherein binding to one or more Fc ligands is altered.

23. An antibody according to claim 1, wherein said Fc variant has a FcγRIIIa-fold:FcγRIIb-fold ratio greater than 1.

24. An antibody according to claim 23, wherein said Fc variant has a FcγRIIIa-fold:FcγRIIb-fold ratio greater than approximately 11:1.

25. An antibody according to claim 24, wherein said Fc variant has a FcγRIIIa-fold:FcγRIIb-fold ratio between approximately 11:1 and approximately 86:1.

26. An antibody according to claim 23, wherein said Fc variant portion comprises at least one substitution at a position corresponding to a position of the human sequence selected from the group consisting of: 234, 235, 239, 240, 264, 296, 330, and I332.

27. An antibody according to claim 26, wherein said Fc variant comprises at least one substitution selected from the group consisting of: 234Y, 234I, 235I, 239D, 239E, 239N, 239Q, 240A, 240M, 264I, 264Y, 296Q, 330L, 330Y, 330I, 332D, and 332E.

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28. An antibody according to claim 27, wherein said Fc variant is selected from the group consisting of 332E, 264I/332E, 239E/332E, 239Q/332E, 296Q, 330L, 330Y, 332D, 239D, 239D/332E, 330Y/332E, 264I/330Y/332E, 330L/332E, 264I/330L/332E, 234Y, 234I, 235I, 240A, 240M, 264Y, 330I, 239D/330L/332E, 239D/298A/332E, 239N/298A/332E, 239D/264I/332E, 239D/264I/298A/332E, and 239D/264I/330L/332E.

29. An antibody according to claim 26, wherein said Fc variant further comprises one or more substitutions at a position selected from the group consisting of 256, 270, 290, 298, 312, 322, 326, 329, 331, 333, 334, and 339.

30. An antibody according to claim 1, wherein said Fc variant binds to at least one FcγR with reduced affinity relative to the parent Fc polypeptide.

31. An antibody according to claim 30, wherein said FcγR is FcγRIIIa.

32. An antibody according to claim 31, wherein said Fc variant comprises at least one substitution selected from the group consisting of 234D, 234N, 234Q, 234T, 234H, 234V, 234F, 235N, 235Q, 235T, 235H, 235V, 235F, 239E, 239N, 239Q, 239F, 239H, 239Y, 240A, 240T, 241W, 241L, 241Y, 241E, 241R, 243W, 243L 243Y, 243R, 243Q, 244H, 245A, 247V, 247G, 262I, 262A, 262T, 262E, 263I, 263A, 263T, 263M, 264L, 264I, 264W, 264T, 264R, 264F, 264M, 264E, 265G, 265N, 265Q, 265Y, 265F, 265V, 265I, 265L, 265H, 265T, 266A, 266T, 266M, 267Q, 267L, 269H, 269Y, 269F, 269R, 296E, 296Q, 296D, 296N, 296S, 296T, 296L, 296I, 296H, 297S, 297D, 297E, 298H, 299I, 299L, 299A, 299S, 299V, 299H, 299F, 299E, 313F, 325Q, 325L, 325I, 325D, 325E, 325A, 325V, 325H, 327N, 327L, 328M, 328E, 328N, 328Q, 328F, 328H, 328A, 329F, 330L, 330V, 330F, 330R, 330H, 332N, 332Q, 332T, 332H, 332Y, and 332A

33. An antibody according to claim 31, wherein said Fc variant is selected from the group consisting of 264L, 241W, 241L, 243W, 243L, 241L/243L/262I/264I, 241W/243W, 241W/243W/262A/264A, 241L/262I, 243L/262I/264W, 241Y/243Y/262T/264T, 241E/243R/262E/264R, 241E/243Q/262T/264E, 241R/243Q/262T/264R, 241E/243Y/262T/264R, 328M, 328E, 328F, 244H, 245A, 247V, 313F, 244H/245A/247V, 247G, 241E/243R/262E/264R/332E, 241E/243Y/262T/264R/332E, 265G, 265N, 239E/265G, 239E/265N, 239E/265Q, 296E, 296Q, 299I, 327N, 267Q/327S, 267L/327S , 327L, 329F, 330L, 297S, 297D, 297S/332E, 332N, 332Q, 264F, 263I, 299A, 299S, 299V, 325Q, 325L, 325I, 239N, 239F, 239N/332N, 239N/332Q, 239Q/332N, 239Q/332Q, 296D, 296N, 234D, 234N, 234Q, 234T, 234H, 234V, 234F, 235N, 235Q, 235T, 235H, 235V, 235F, 239H, 239Y,

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240A, 263T, 263M, 264M, 266A, 266T, 266M, 269H, 269Y, 269F, 269R, 296S, 296T, 296L, 296I, 298H, 299H, 330V, 330F, 330R, 330H, 325D, 325E, 325A, 325V, 325H, 328E/332E, 328N/332E, 328Q/332E, 328H/332E, 328A, 332T, 332H, 332Y, and 332A.

34. An antibody comprising a variant of a parent Fc polypeptide comprising at least one amino acid modification in the Fc region of said parent Fc polypeptide, wherein said Fc variant modulates effector function as compared to the parent Fc polypeptide.

35. An antibody according to claim 34, wherein said effector function is ADCC.

36. An antibody according to claim 35, wherein said Fc variant improves ADCC as compared to said parent Fc polypeptide.

37. An antibody according to claim 36, wherein said ADCC improvement is approximately 5-fold greater than that of said parent Fc polypeptide.

38. An antibody according to claim 37, wherein said ADCC improvement is between approximately 5-fold and 50-fold greater than that of said parent Fc polypeptide.

39. An antibody according to claim 37, wherein said Fc variant portion comprises at least one substitution at a position corresponding to a position of the human sequence selected from the group consisting of: 234, 235, 239, 240, 243, 264, 266, 328, 330, 332, and 325.

40. An antibody according to claim 39, wherein said Fc variant comprises at least one substitution selected from the group consisting of 234E, 234Y, 234I, 235D, 235S, 235Y, 235I, 239D, 239E, 239N, 239Q, 239T, 240I, 240M, 243L, 264I, 264T, 264Y, 266I, 328M, 328I, 328Q, 328D, 328V, 328T, 330Y, 330L, 330I, 332D, 332E, 332N, 332Q, and 325T.

41. An antibody according to claim 39, wherein said Fc variant is selected from the group consisting of 264I, 243L/264I, 328M, 332E, 328M/332E, 264I/332E, 298A/332E, 239E/332E, 239Q/332E, 239E, 330Y, 332D, 328I/332E, 328Q/332E, 264T, 240I, 266I, 239D, 239D/332D, 239D/332E, 239D/332N, 239D/332Q, 239E/332D, 239E/332N, 239E/332Q, 239N/332D, 239N/332E, 239Q/332D, 330Y/332E, 264I/330Y/332E, 330L/332E, 264I/330L/332E, 234E, 234Y, 234I, 235D, 235S, 235Y, 235I, 239T, 240M, 264Y, 330I, 325T, 328D/332E, 328V/332E, 328T/332E, 328I/332E, 239E/264I/332E, 239Q/264I/332E, 239E/264I/330Y/332E,

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239D/330Y/332E, 239N/330Y/332E, 239D/330L/332E, 239N/330L/332E, 264I/298A/332E, 239D/298A/332E, 239N/298A/332E, 239D/264I/332E, 239D/264I/298A/332E, and 239D/264I/330L/332E.

42. An antibody according to claim 39, wherein said Fc variant further comprises at least one substitution at a position selected from the group consisting of 256, 270, 290, 298, 312, 322, 326, 329, 331, 333, 334, and 339.

43. An antibody according to claim 39, wherein said parent Fc polypeptide is substantially human, substantially mouse, substantially rat, or substantially monkey.

44. An antibody according to claim 35, wherein said Fc variant reduces ADCC as compared to said parent Fc polypeptide.

45. An antibody according to claim 44, wherein said Fc variant comprises at least one substitution selected from the group consisting of 234D, 234N, 234Q, 234T, 234H, 234V, 234F, 235N, 235Q, 235T, 235H, 235V, 235F, 239E, 239N, 239Q, 239F, 239H, 239Y, 240A, 240T, 241W, 241L, 241Y, 241E, 241R, 243W, 243L 243Y, 243R, 243Q, 244H, 245A, 247V, 247G, 262I, 262A, 262T, 262E, 263I, 263A, 263T, 263M, 264L, 264I, 264W, 264T, 264R, 264F, 264M, 264E, 265G, 265N, 265Q, 265Y, 265F, 265V, 265I, 265L, 265H, 265T, 266A, 266T, 266M, 267Q, 267L, 269H, 269Y, 269F, 269R, 296E, 296Q, 296D, 296N, 296S, 296T, 296L, 296I, 296H, 297S, 297D, 297E, 298H, 299I, 299L, 299A, 299S, 299V, 299H, 299F, 299E, 313F, 325Q, 325L, 325I, 325D, 325E, 325A, 325V, 325H, 327N, 327L, 328M, 328E, 328N, 328Q, 328F, 328H, 328A, 329F, 330L, 330V, 330F, 330R, 330H, 332N, 332Q, 332T, 332H, 332Y, and 332A.

46. An antibody according to claim 44, wherein said Fc variant is selected from the group consisting of 264L, 241W, 241L, 243W, 243L, 241L/243L/262I/264I, 241W/243W, 241W/243W/262A/264A, 241L/262I, 243L/262I/264W, 241Y/243Y/262T/264T, 241E/243R/262E/264R, 241E/243Q/262T/264E, 241R/243Q/262T/264R, 241E/243Y/262T/264R, 328M, 328E, 328F, 244H, 245A, 247V, 313F, 244H/245A/247V, 247G, 241E/243R/262E/264R/332E, 241E/243Y/262T/264R/332E, 265G, 265N, 239E/265G, 239E/265N, 239E/265Q, 296E, 296Q, 299I, 327N, 267Q/327S, 267L/327S , 327L, 329F, 330L, 297S, 297D, 297S/332E, 332N, 332Q, 264F, 263I, 299A, 299S, 299V, 325Q, 325L, 325I, 239N, 239F, 239N/332N, 239N/332Q, 239Q/332N, 239Q/332Q, 296D, 296N, 234D, 234N, 234Q, 234T, 234H, 234V, 234F, 235N, 235Q, 235T, 235H, 235V, 235F, 239H, 239Y, 240A, 263T, 263M, 264M, 266A, 266T, 266M, 269H, 269Y, 269F, 269R, 296S, 296T, 296L,

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296I, 298H, 299H, 330V, 330F, 330R, 330H, 325D, 325E, 325A, 325V, 325H, 328E/332E, 328N/332E, 328Q/332E, 328H/332E, 328A, 332T, 332H, 332Y, and 332A.

47. An antibody comprising an aglycosylated Fc variant of a parent Fc polypeptide comprising at least one amino acid modification in the Fc region of said parent Fc polypeptide, wherein said aglycosylated Fc variant has improved stability, solubility, or binding affinity to an Fc ligand relative to the aglycosylated form of said parent Fc polypeptide.

48. An antibody according to claim 47, wherein said aglycosylated Fc variant has improved binding affinity to an Fc ligand as compared to the aglycosylated form of the parent Fc polypeptide.

49. An antibody according to claim 48, wherein said Fc ligand is an FcγR.

50. An antibody according to claim 49, wherein said FcγR is FcγRIIIa.

51. An antibody according to claim 48, wherein said improved binding affinity is within 0.4-fold of the glycosylated form of the parent Fc polypeptide.

52. An antibody according to claim 47, wherein said Fc variant portion comprises at least one substitution at a position corresponding to a position of the human sequence selected from the group consisting of: 239, 241, 243, 262, 264, 265, 296, 297, 330, and 332.

53. An antibody according to claim 52, wherein said Fc variant comprises one or more substitutions selected from the group consisting of 239D, 239E, 241Y, 243Y, 262T, 264T, 264E, 265Y, 265H, 296N, 297D, 330Y, and 332E.

54. An antibody according to claim 52, wherein said Fc variant is selected from the group consisting of 297D/332E, 241Y/243Y/262T/264T/297D/332E, 239D/297D/332E, 239E/297D/332E, 239D/265Y/297D/332E, 239D/265H/297D/332E, 264E/297D/332E, 296N/297D/332E, and 297D/330Y/332E.

55. An antibody according to claim 53, wherein said aglycosylated Fc variant portion comprises at least one substitution at a position corresponding to a position of the human sequence

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selected from the group consisting of: 256, 270, 290, 298, 312, 322, 326, 329, 331, 333, 334, and 339.

56. An antibody according to claim 1 comprising a Fc fusion comprising said Fc variant.

57. An antibody according to claim 1 or 56, wherein said antibody further comprises an engineered glycoform.

58. An antibody according to claim 57, wherein said engineered glycoform improves effector function.

59. A pharmaceutical composition comprising an antibody according to claim 1 and a pharmaceutically acceptable carrier.

60. A method of treating a mammal in need of said treatment, comprising administering an antibody of claim 1.

61. An antibody comprising an Fc variant, said Fc variant comprises at least one substitution selected from the group consisting of: 332D, 332E, 332N, or 332Q, wherein said positions corresponding to the human sequence position, wherein said antibody has specificity for a target antigen selected from the group consisting of CD20, CD22, CD33, CD52, Her2/neu, EGFR, EpCAM, MUC1, GD3, CEA, CA 125, HLA-DR, TNFalpha, and VEGF.

62. An antibody comprising an Fc variant, said Fc variant comprises at least one substitution selected from the group consisting of 264I, 264T, or 264Y, wherein said positions corresponding to the human sequence position, wherein said antibody or Fc fusion has specificity for a target antigen selected from the group consisting of CD20, CD22, CD33, CD52, Her2/neu, EGFR, EpCAM, MUC1, GD3, CEA, CA 125, HLA-DR, TNFalpha, and VEGF.

63. An antibody comprising an Fc variant, said Fc variant comprises at least one substitution selected from the group consisting of 239D, 239E, 239N, 239Q, or 239T, wherein said positions corresponding to the human sequence position, wherein said antibody or Fc fusion has specificity for a target antigen selected from the group consisting of CD20, CD22, CD33,

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CD52, Her2/neu, EGFR, EpCAM, MUC1, GD3, CEA, CA 125, HLA-DR, TNFalpha, and VEGF.

64. An antibody comprising an Fc variant, said Fc variant comprises at least one substitution selected from the group consisting of 330Y, 330L, or 330I, wherein said positions corresponding to the human sequence position, wherein said antibody or Fc fusion has specificity for a target antigen selected from the group consisting of CD20, CD22, CD33, CD52, Her2/neu, EGFR, EpCAM, MUC1, GD3, CEA, CA 125, HLA-DR, TNFalpha, and VEGF.

65. An antibody comprising an Fc variant, said Fc variant comprises at least one substitution selected from the group consisting of 240I or 240M, wherein said positions corresponding to the human sequence position, wherein said antibody or Fc fusion has specificity for a target antigen selected from the group consisting of CD20, CD22, CD33, CD52, Her2/neu, EGFR, EpCAM, MUC1, GD3, CEA, CA 125, HLA-DR, TNFalpha, and VEGF.

66. An antibody comprising an Fc variant, said Fc variant comprises a substitution consisting of 297D, wherein said position corresponds to the human sequence position, wherein said antibody or Fc fusion has specificity for a target antigen selected from the group consisting of CD20, CD22, CD33, CD52, Her2/neu, EGFR, EpCAM, MUC1, GD3, CEA, CA 125, HLA-DR, TNFalpha, and VEGF.

67. An antibody according to claims 61, 62, 63, 64, 65 or 66 wherein said Fc variant additionally comprises the substitutions 298A.

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Figure 1

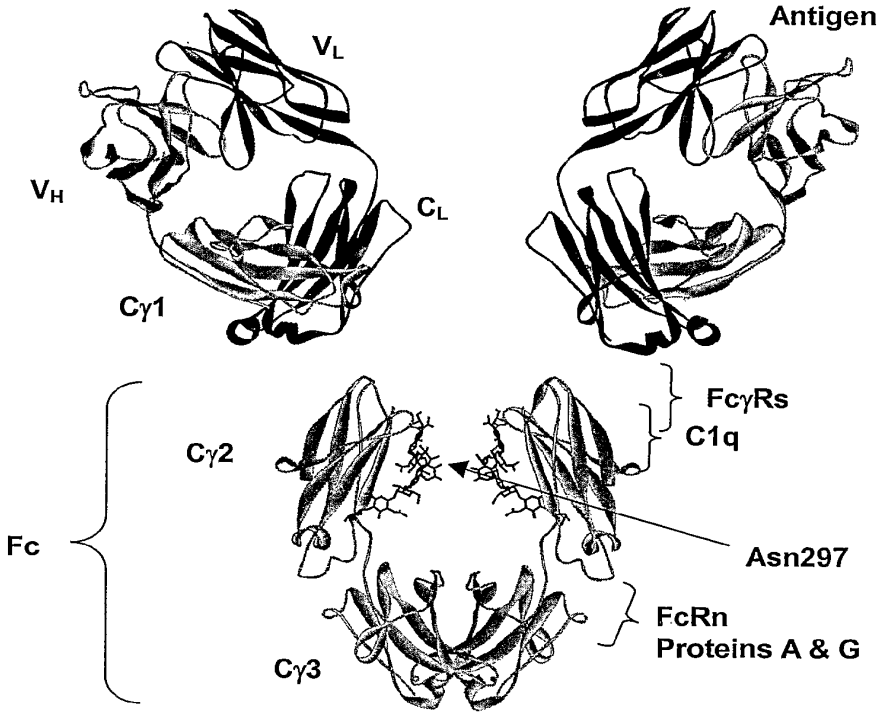

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Figure 2

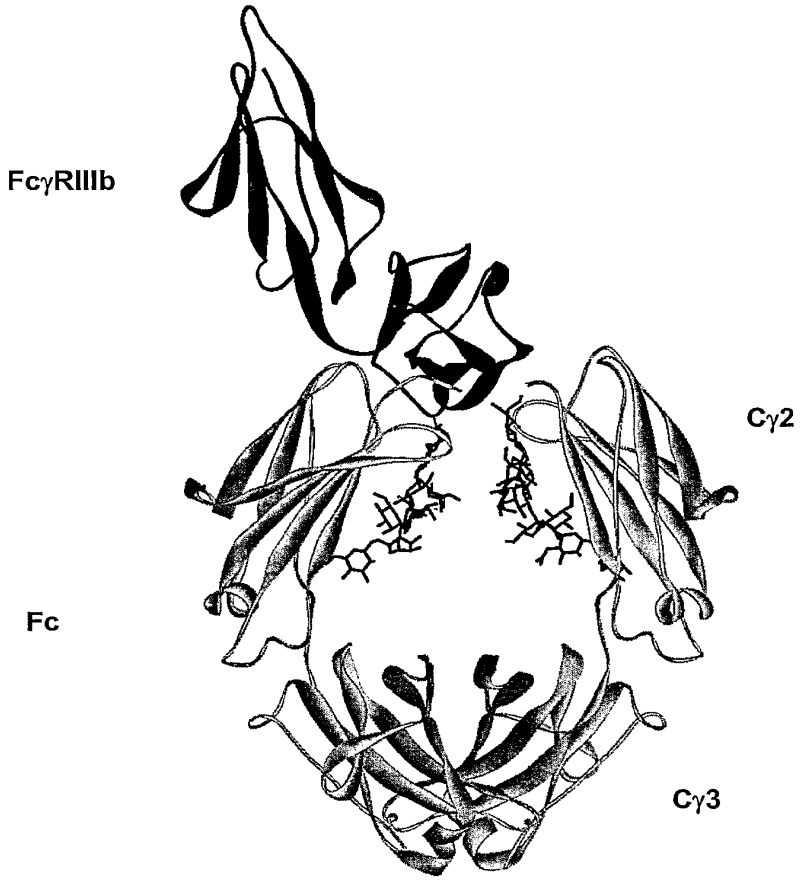

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Figure 3

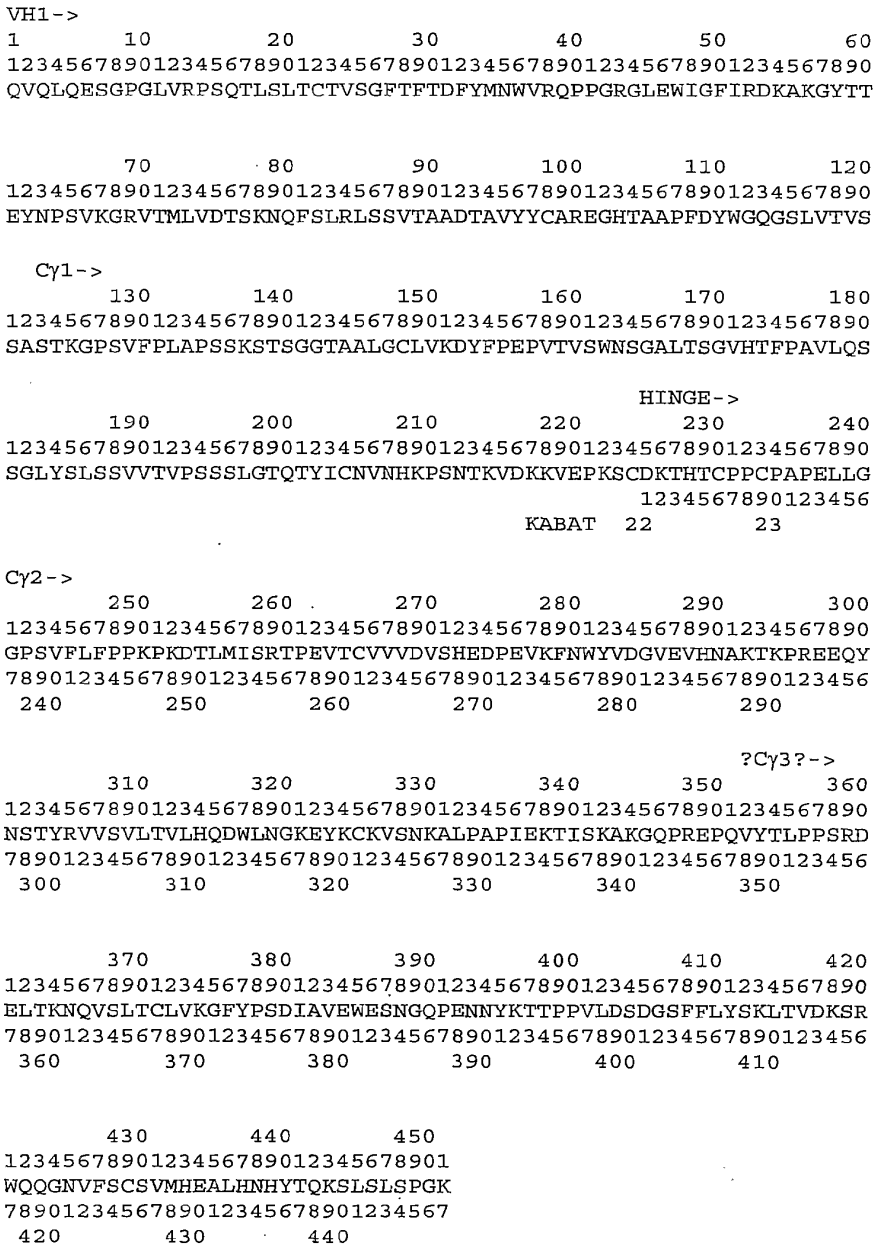

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Figure 4

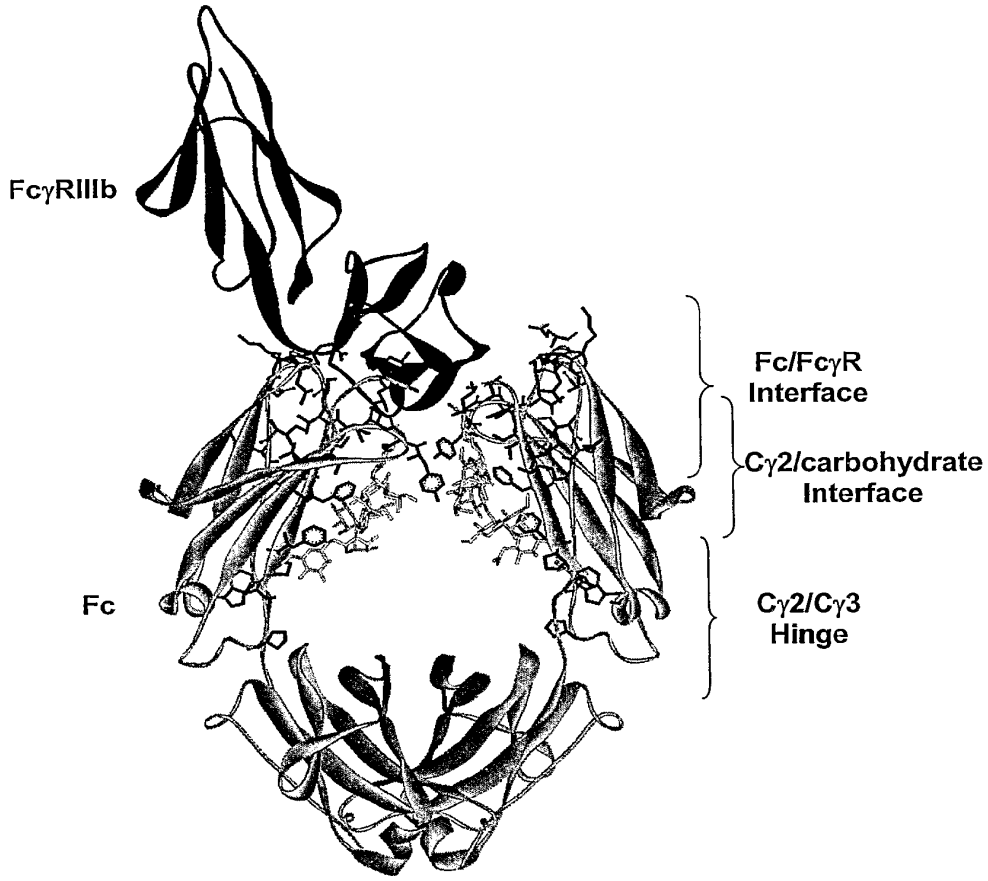

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Figure 5

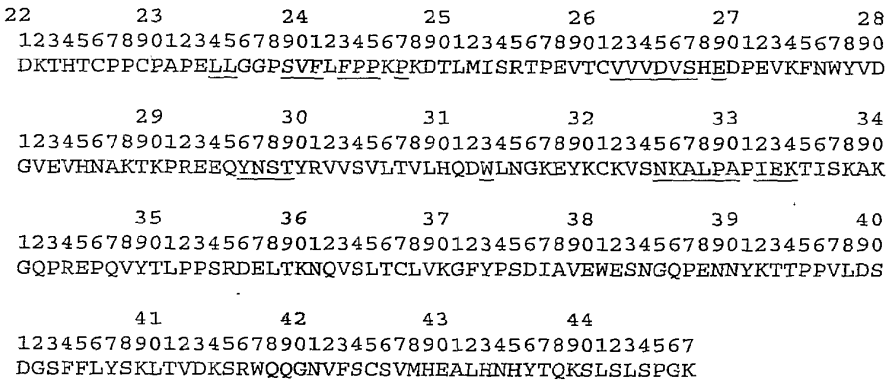

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Figure 6

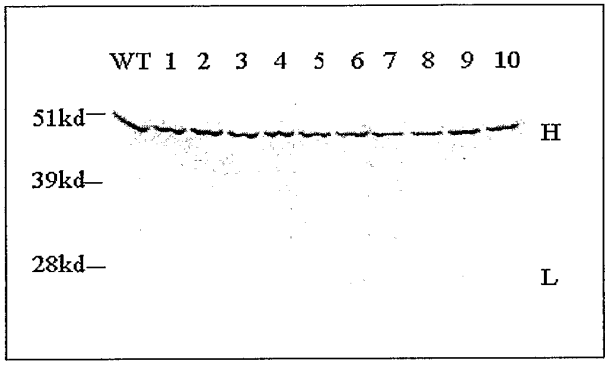

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Figure 7

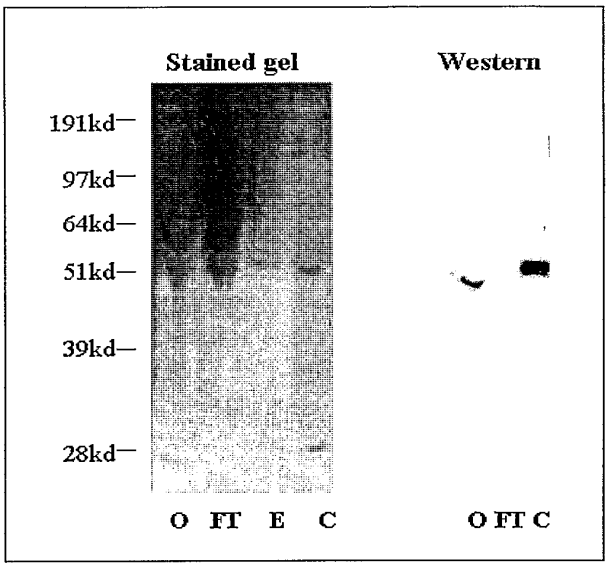

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Figure 8

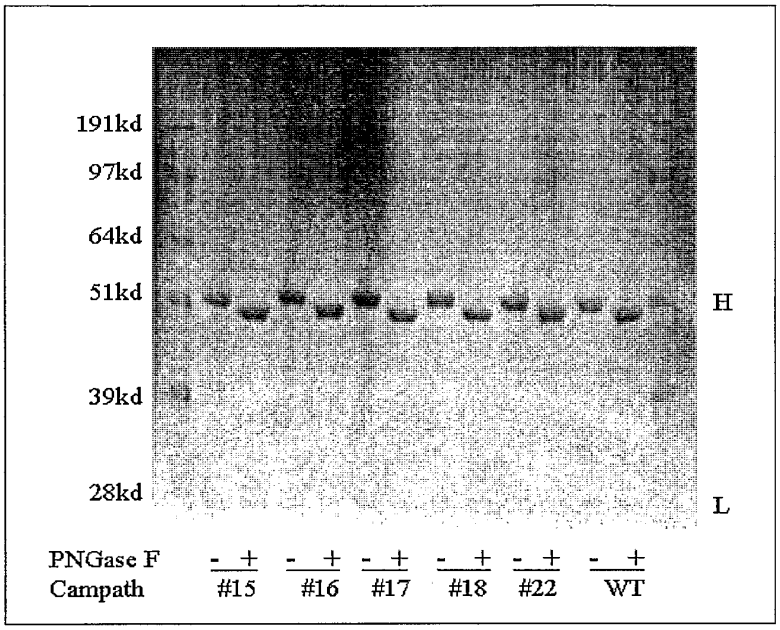
191kd
97kd
64kd
51kd
39kd
28kd
H
L
PNGase F - + - + - + - + - + - +
Campath #15 #16 #17 #18 #22 WT

Figure 9

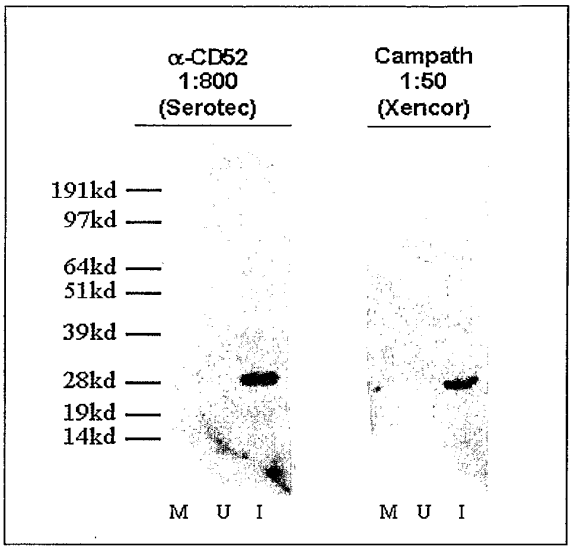
α-CD52
1:800
(Serotec)
Campath
1:50
(Xencor)
191kd
97kd
64kd
51kd
39kd
28kd
19kd
14kd
M U I
M U I

Figure 10

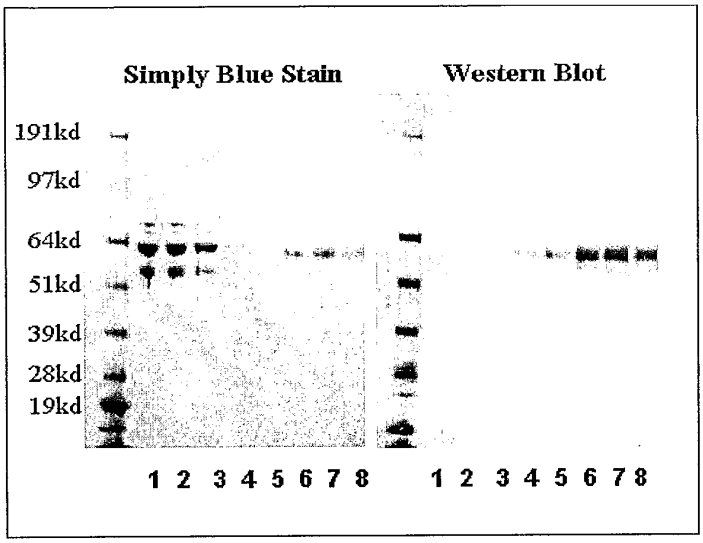

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Figure 11

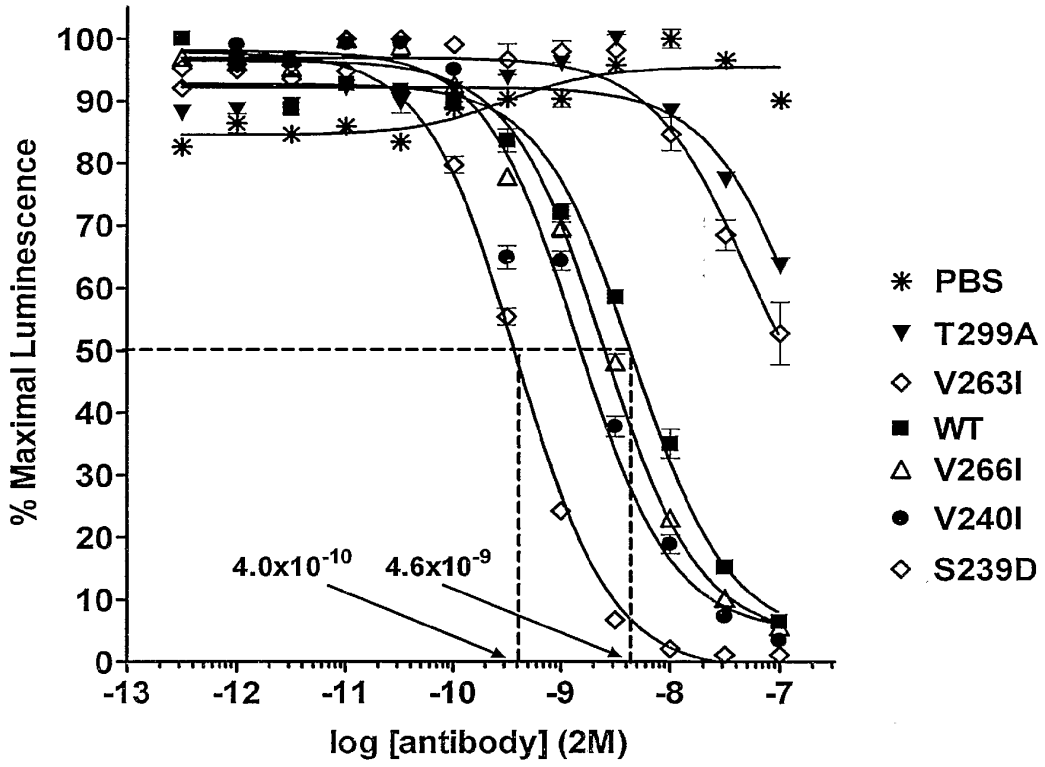

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Figure 12

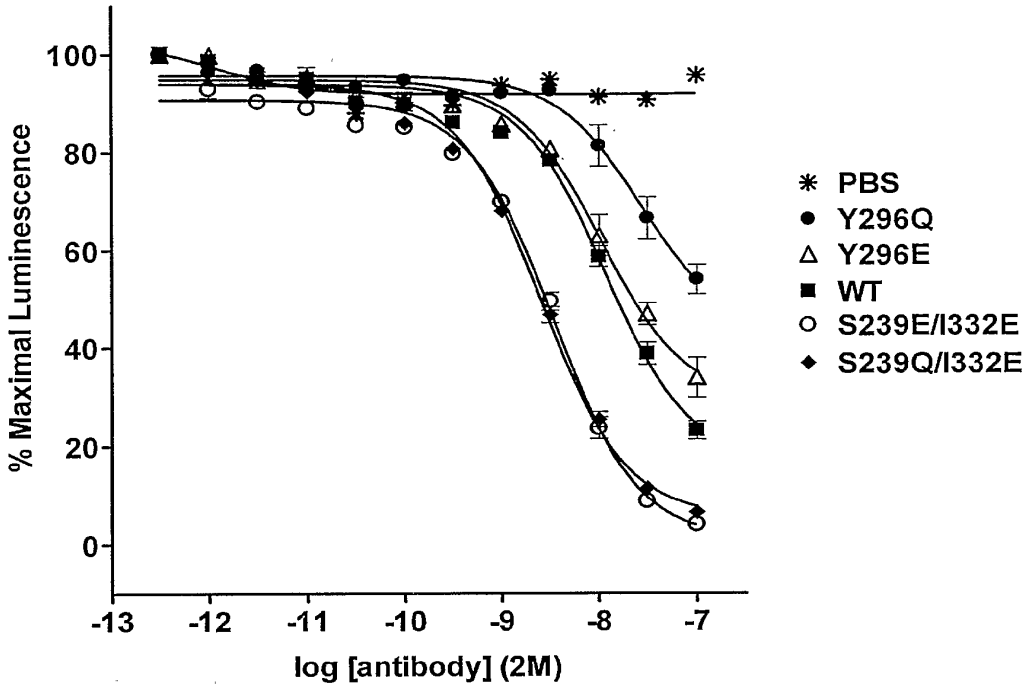

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Figure 13

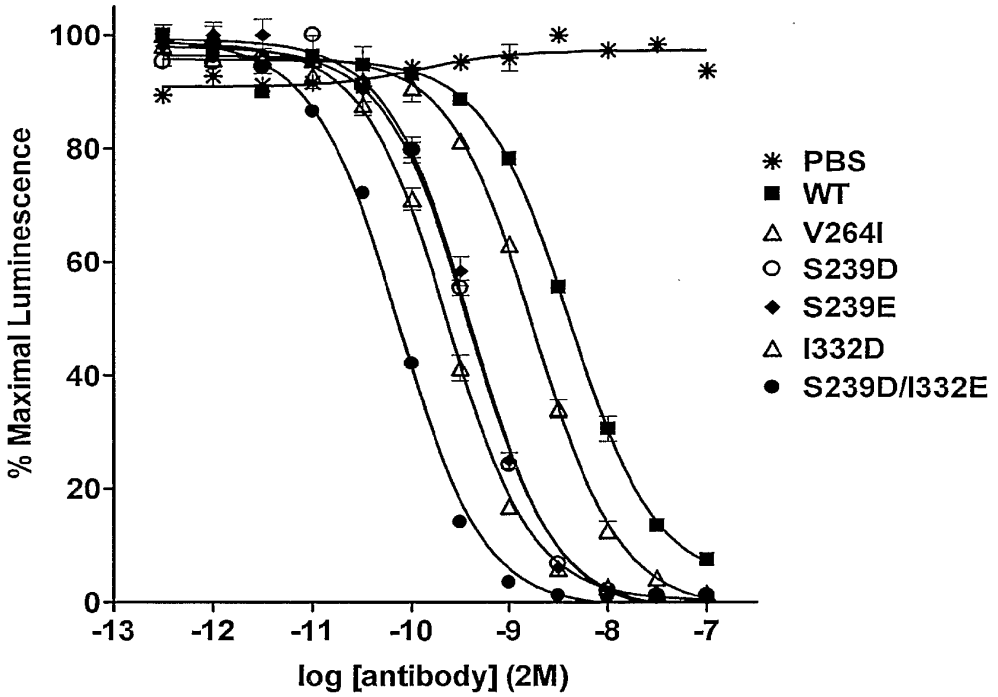
100
80
60
40
20
0
% Maximal Luminescence
-13
-12
-11
-10
-9
-8
-7
log [antibody] (2M)
PBS
WT
V264I
S239D
S239E
I332D
S239D/I332E

Figure 14

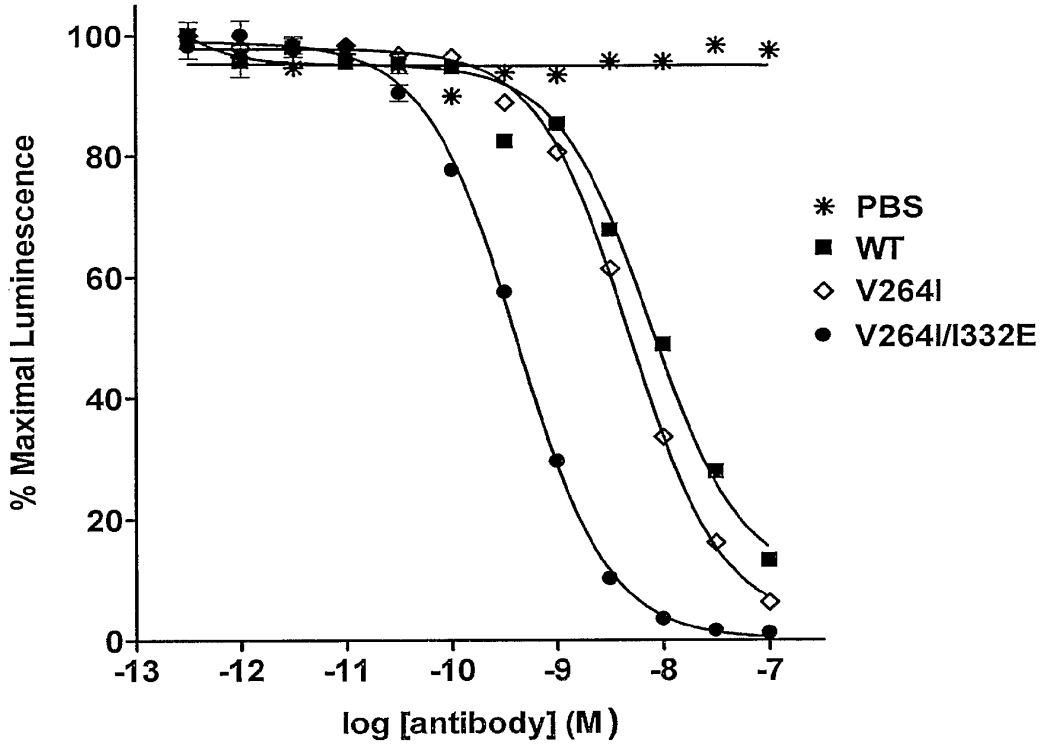
100
80
60
40
20
0
% Maximal Luminescence
-13
-12
-11
-10
-9
-8
-7
log [antibody] (M)
PBS
WT
V264I
V264I/I332E

Figure 15

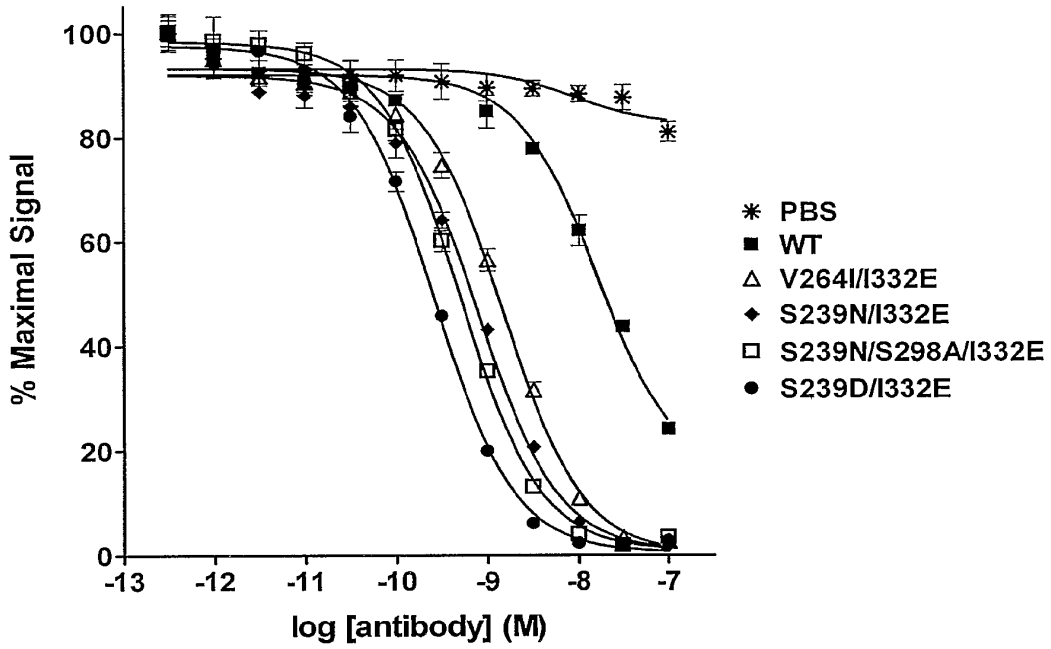
100
80
60
40
20
0
% Maximal Signal
-13
-12
-11
-10
-9
-8
-7
log [antibody] (M)
PBS
WT
V264I/I332E
S239N/I332E
S239N/S298A/I332E
S239D/I332E

Figure 16a

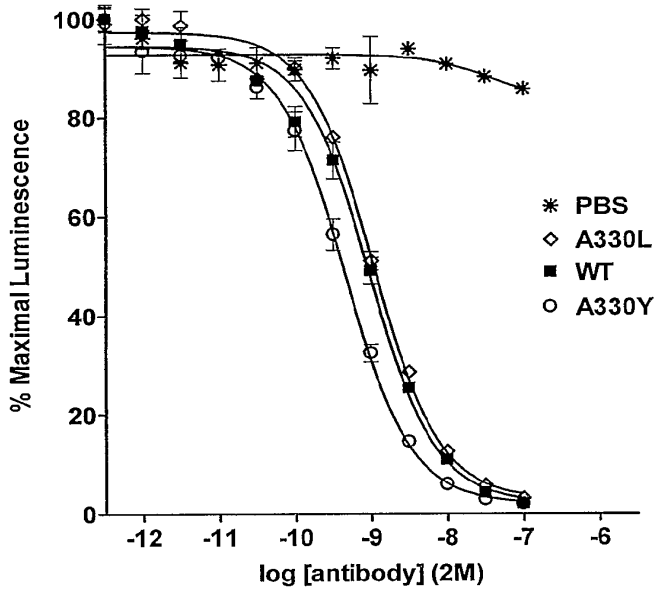

Figure 16b

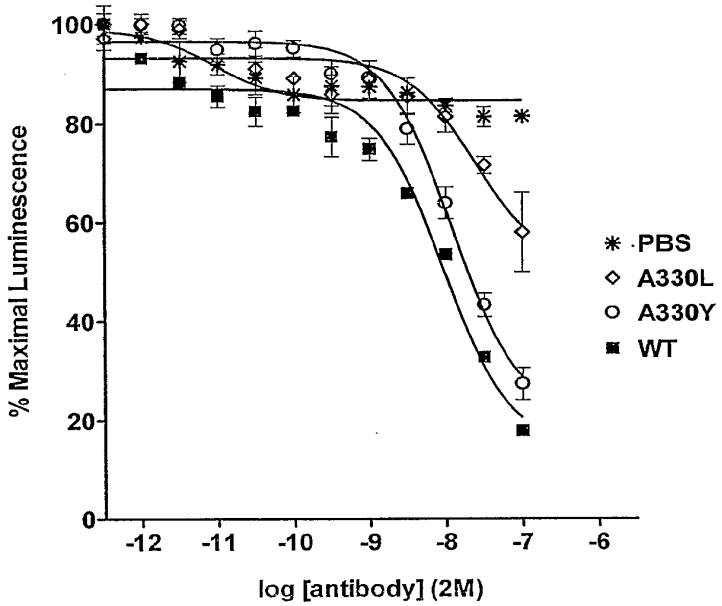

Figure 17

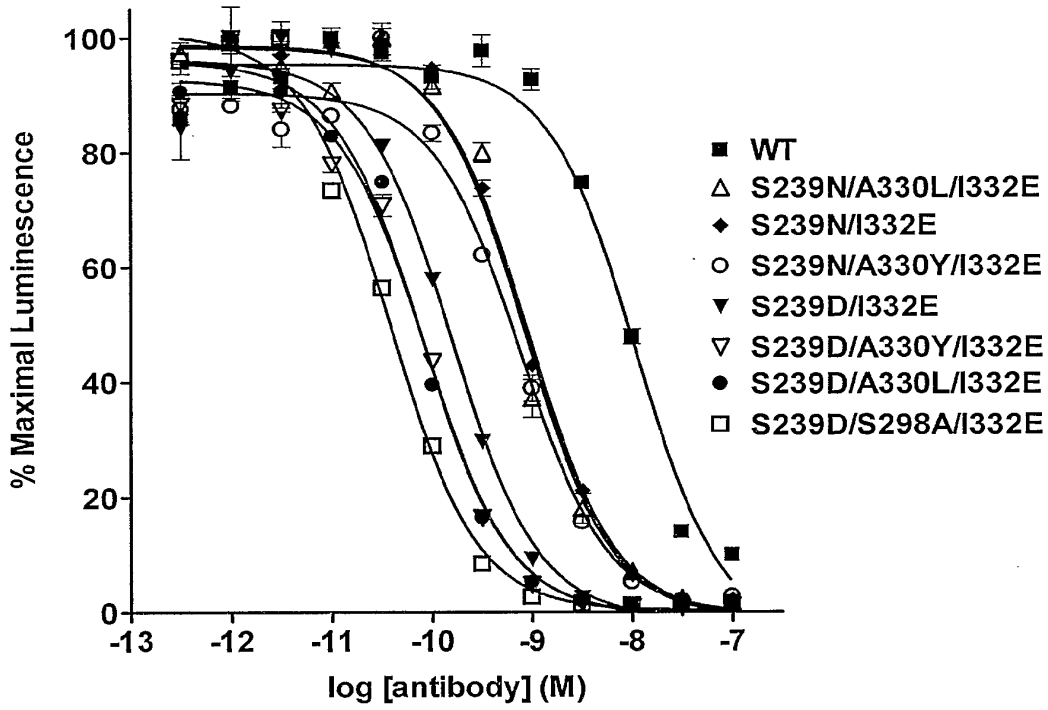
% Maximal Luminescence
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
log [antibody] (M)
WT
S239N/A330L/I332E
S239N/I332E
S239N/A330Y/I332E
S239D/I332E
S239D/A330Y/I332E
S239D/A330L/I332E
S239D/S298A/I332E

Figure 18

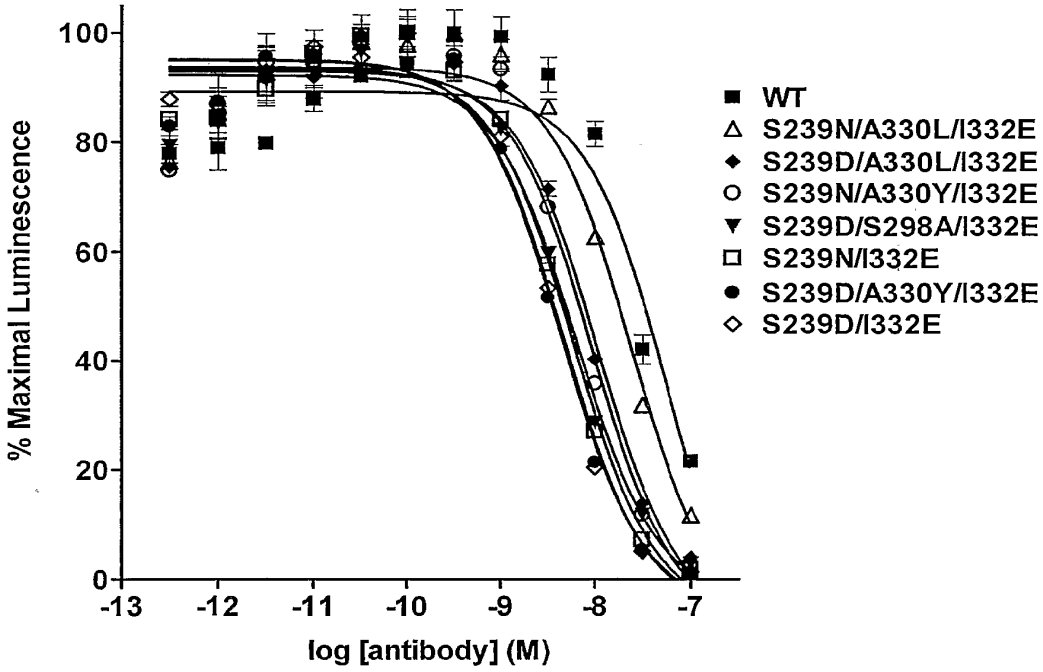
% Maximal Luminescence
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
log [antibody] (M)
WT
S239N/A330L/I332E
S239D/A330L/I332E
S239N/A330Y/I332E
S239D/S298A/I332E
S239N/I332E
S239D/A330Y/I332E
S239D/I332E

Figure 19a

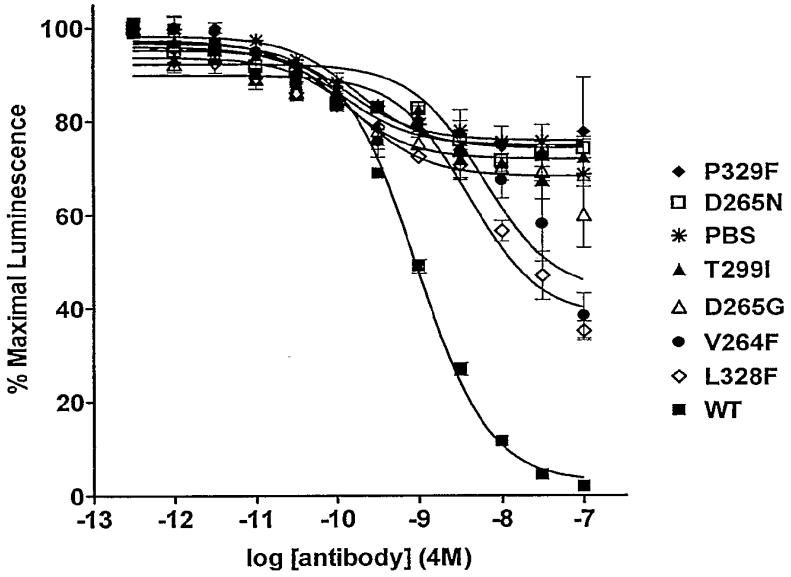

Figure 19b

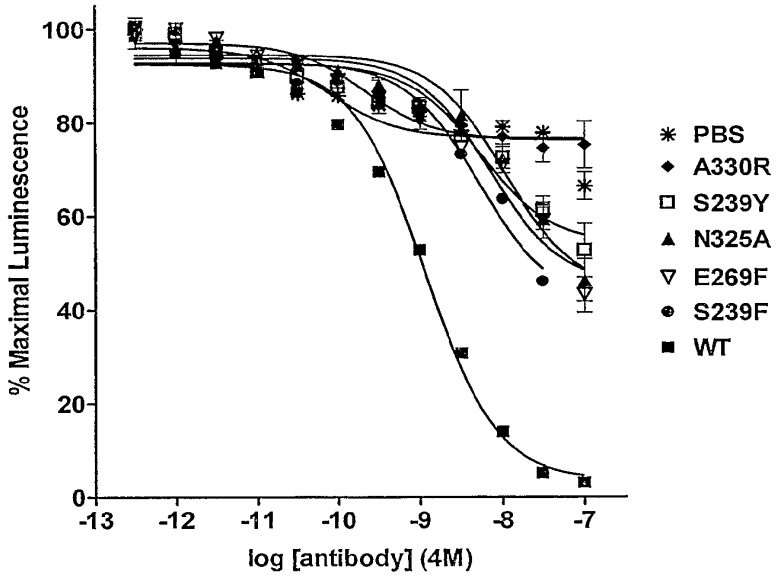

Figure 20

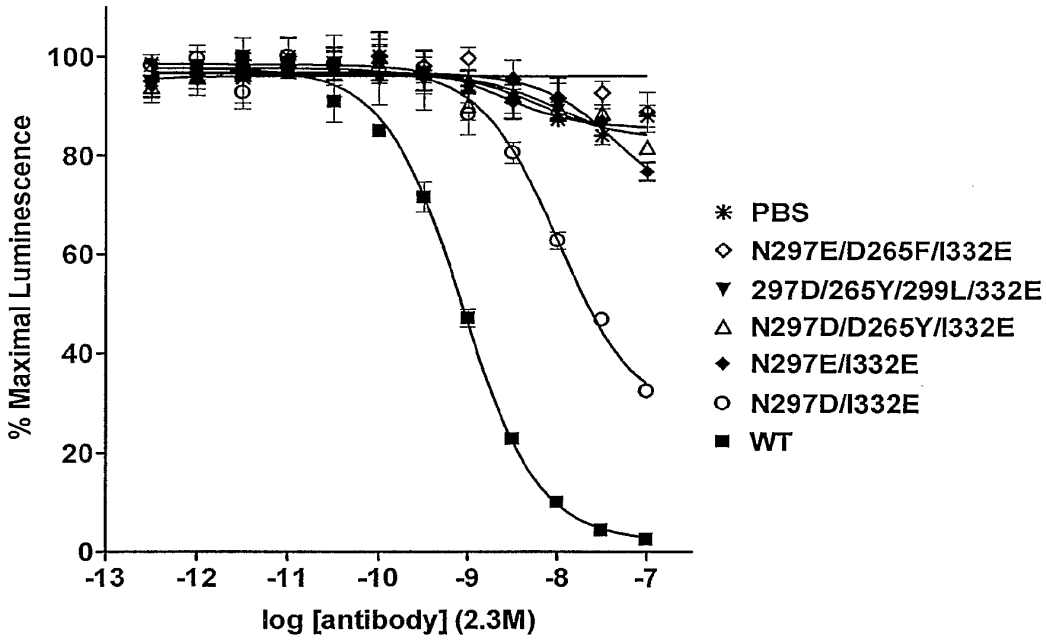

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Figure 21

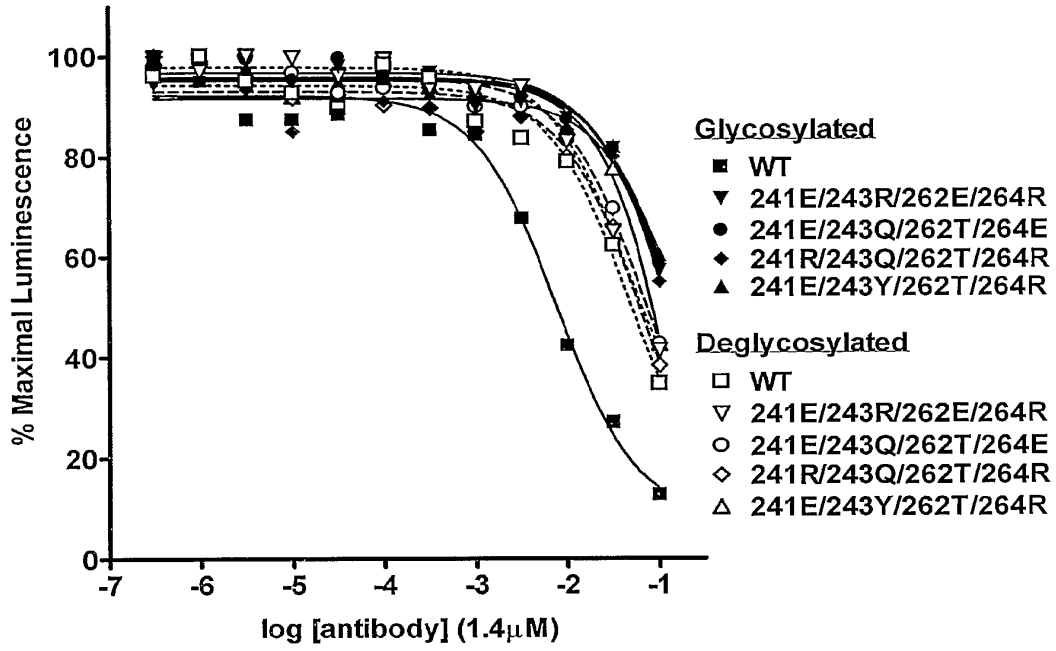

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Figure 22a

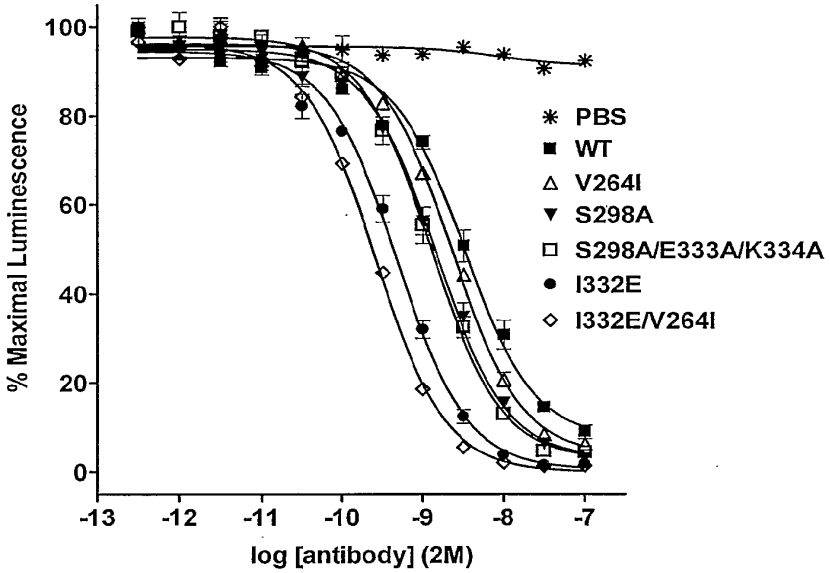

Figure 22b

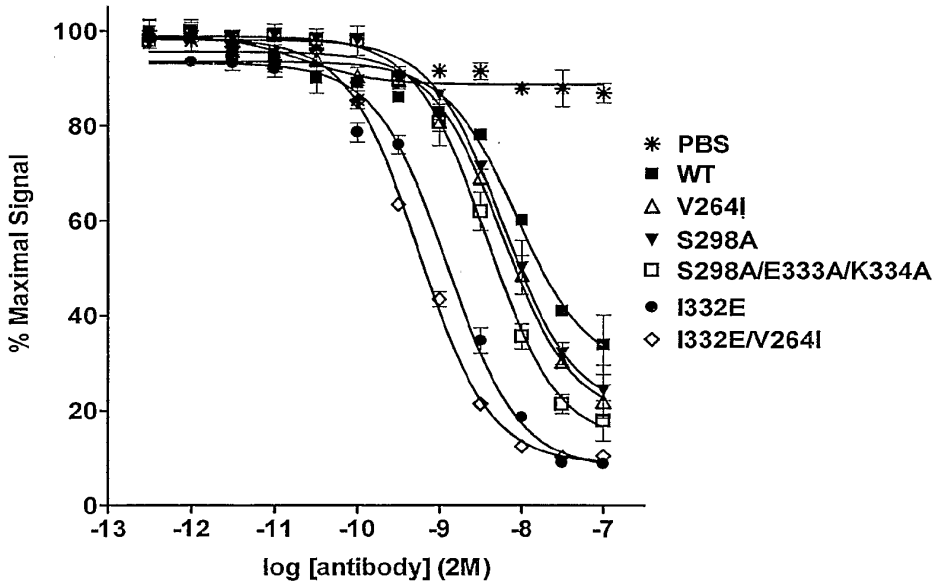

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Figure 23a

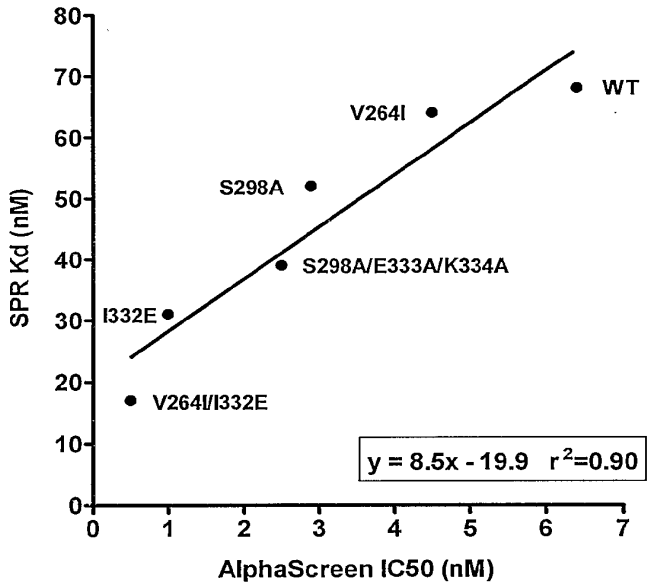

Figure 23b

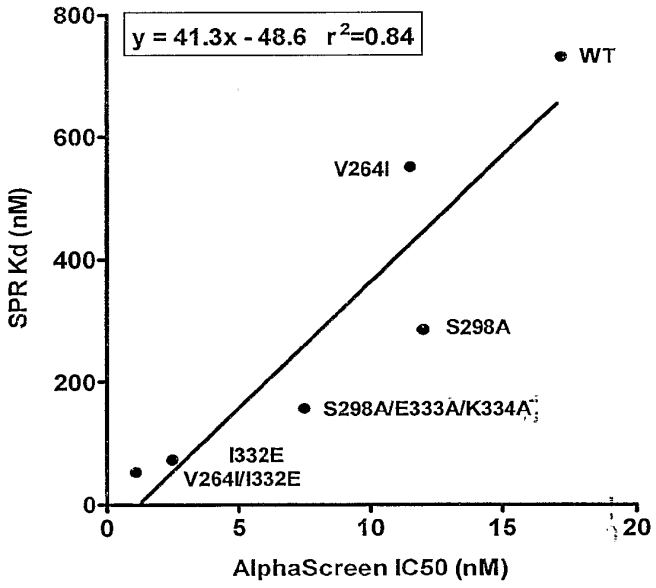

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Figure 23c

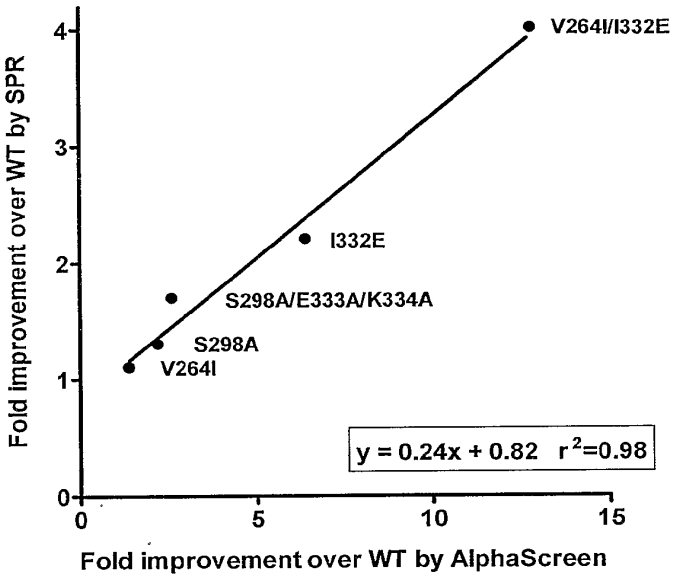

Figure 23d

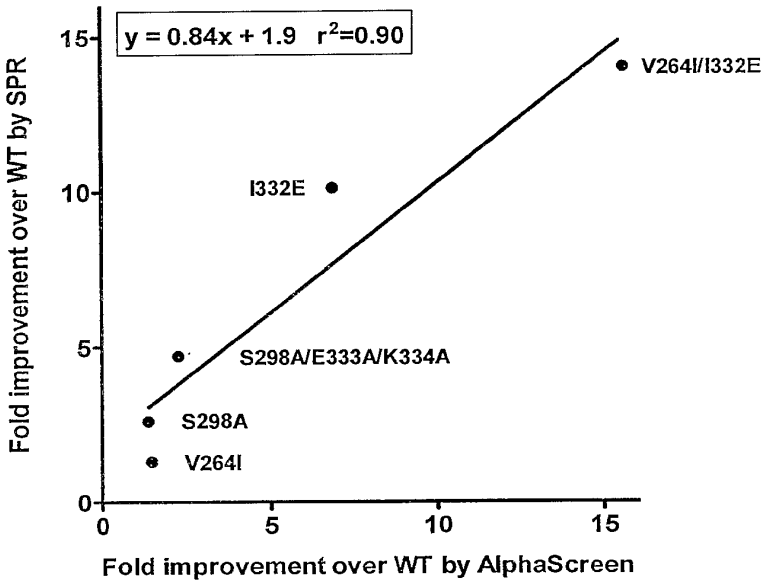

Figure 24a

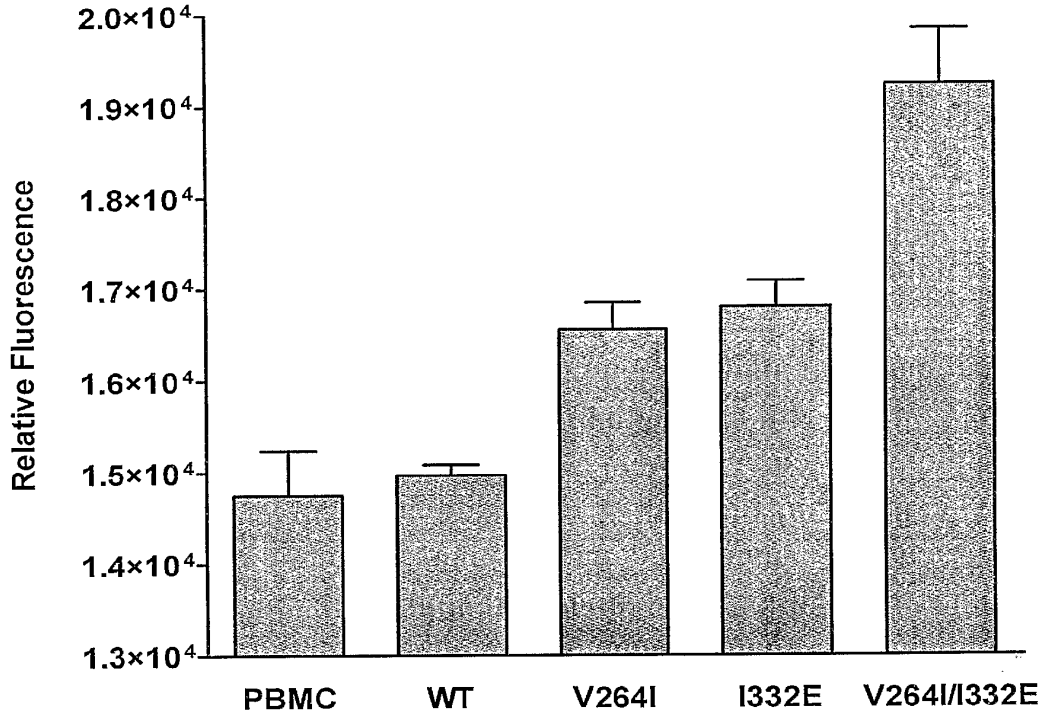

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Figure 24b

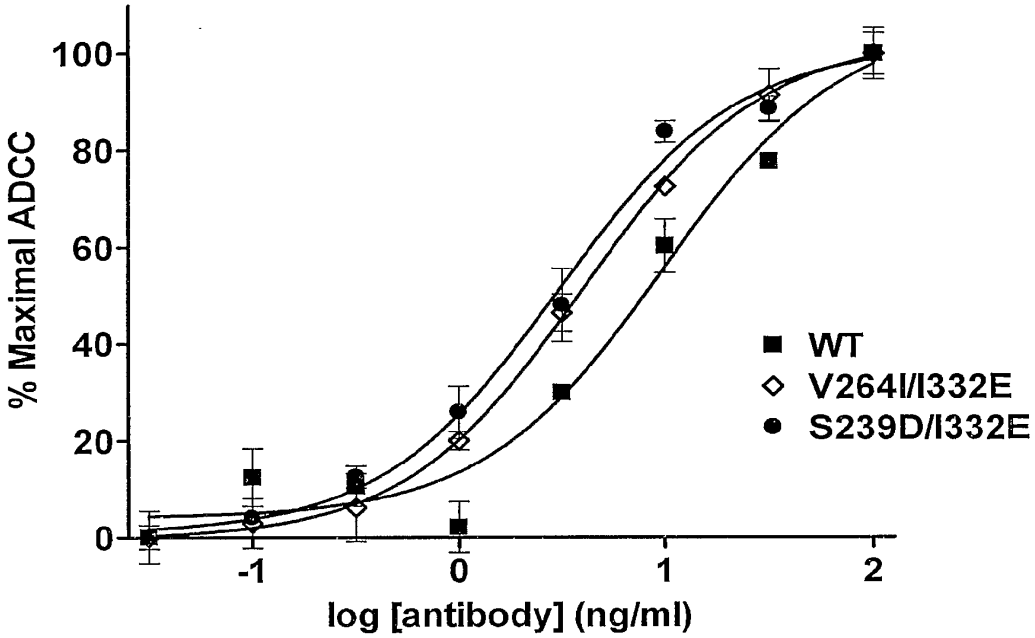

% Maximal ADCC
100
80
60
40
20
0
-1
0
1
2
log [antibody] (ng/ml)
WT
V264I/I332E
S239D/I332E

Figure 25a

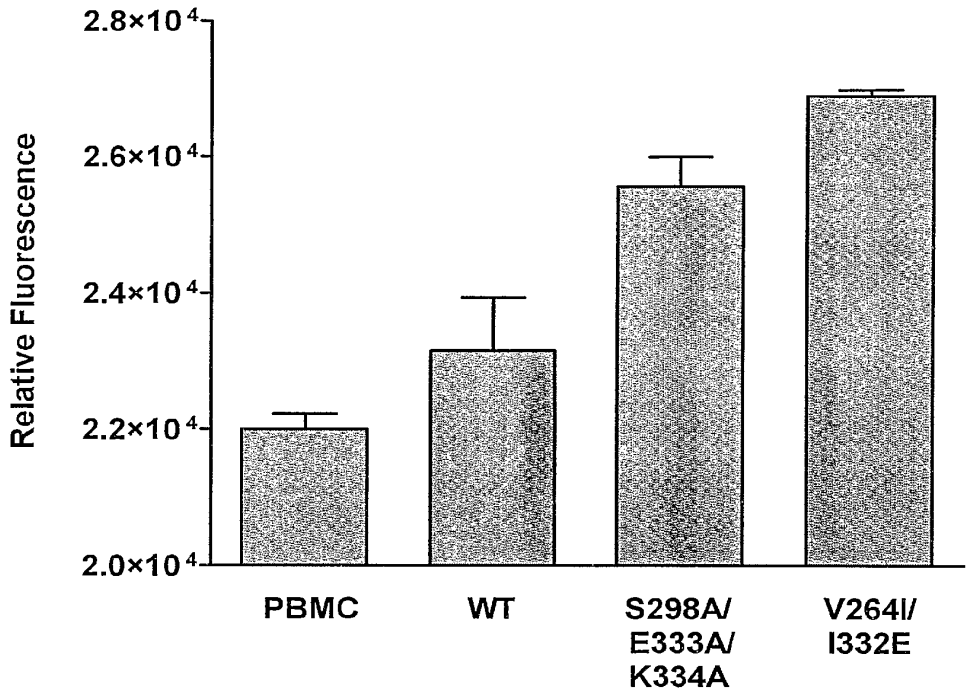

2.8×10 4
2.6×10 4
2.4×10 4
2.2×10 4
2.0×10 4
Relative Fluorescence
PBMC
WT
S298A/
E333A/
K334A
V264I/
I332E

Figure 25b

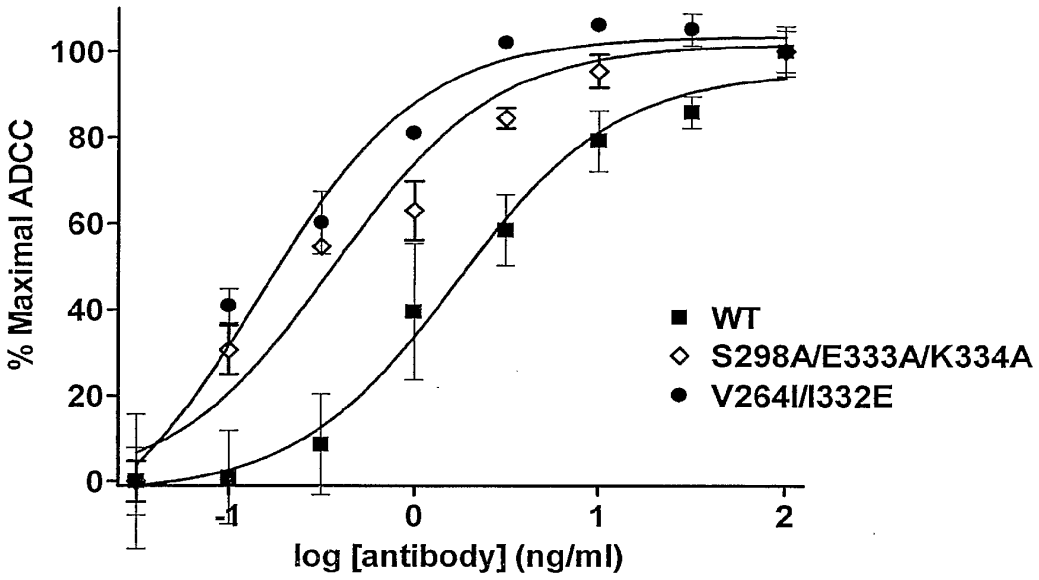
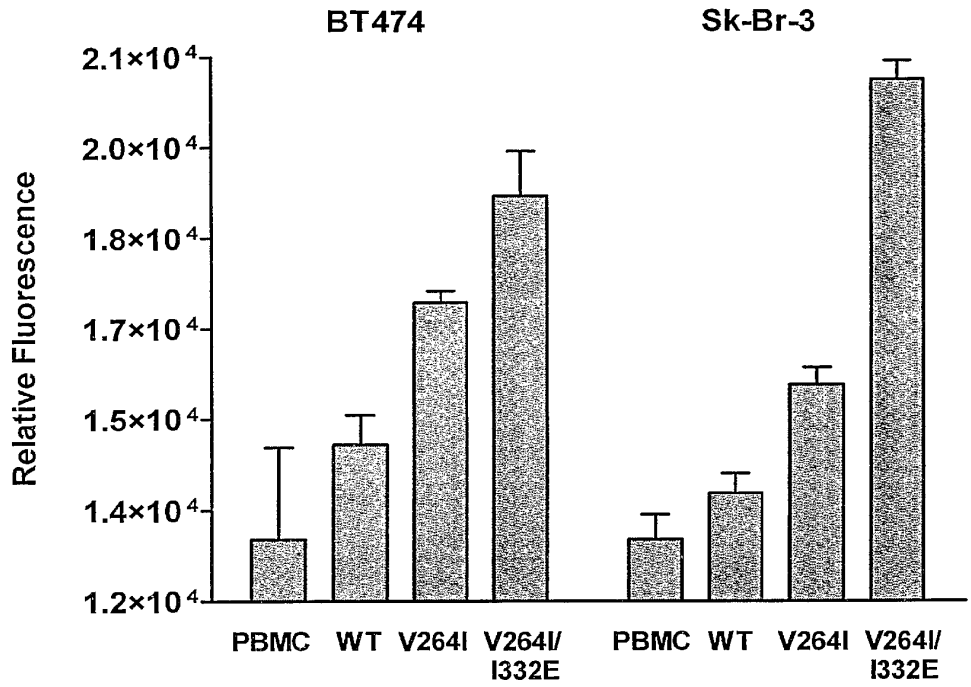

% Maximal ADCC
100
80
60
40
20
0
-1
0
1
2
log [antibody] (ng/ml)
WT
S298A/E333A/K334A
V264I/I332E

Figure 26a


BT474
Sk-Br-3
Relative Fluorescence
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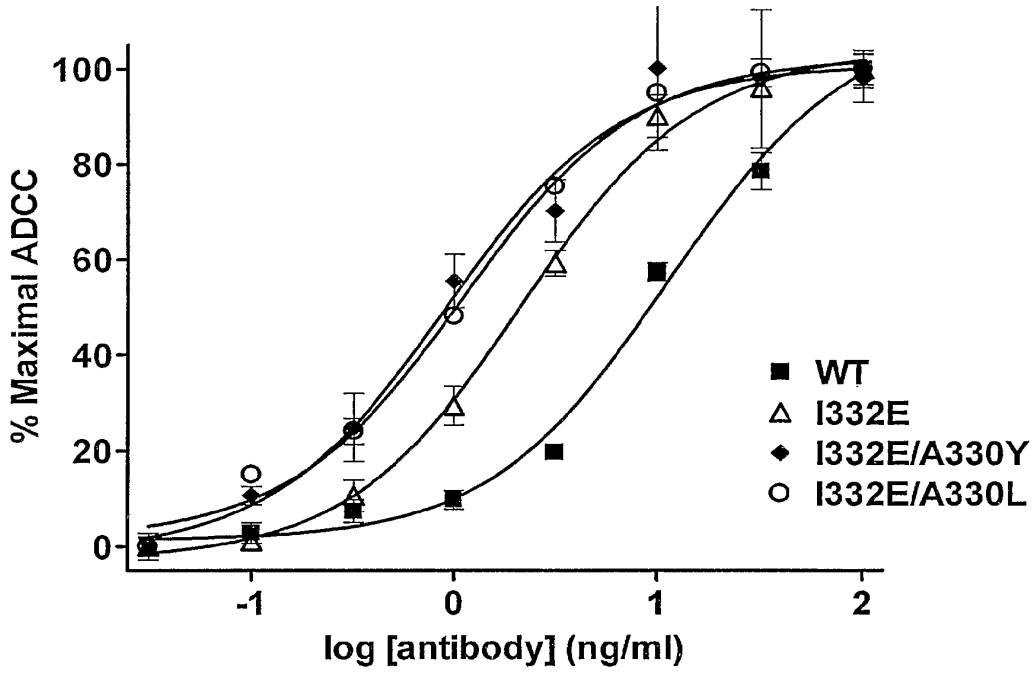
2.0×10 4
1.8×10 4
1.7×10 4
1.5×10 4
1.4×10 4
1.2×10 4
PBMC
WT
V264I
V264I/ I332E
PBMC
WT
V264I
V264I/ I332E

Figure 26b


% Maximal ADCC
100
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60
40
20
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1
2
log [antibody] (ng/ml)
WT
I332E
I332E/A330Y
I332E/A330L

Figure 26c

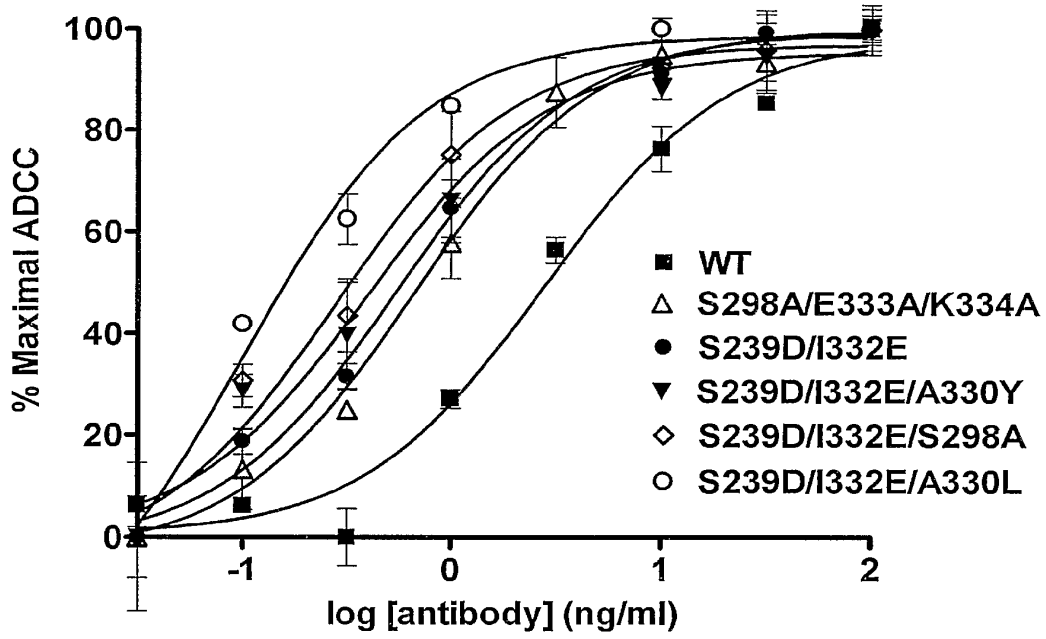

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Figure 27a

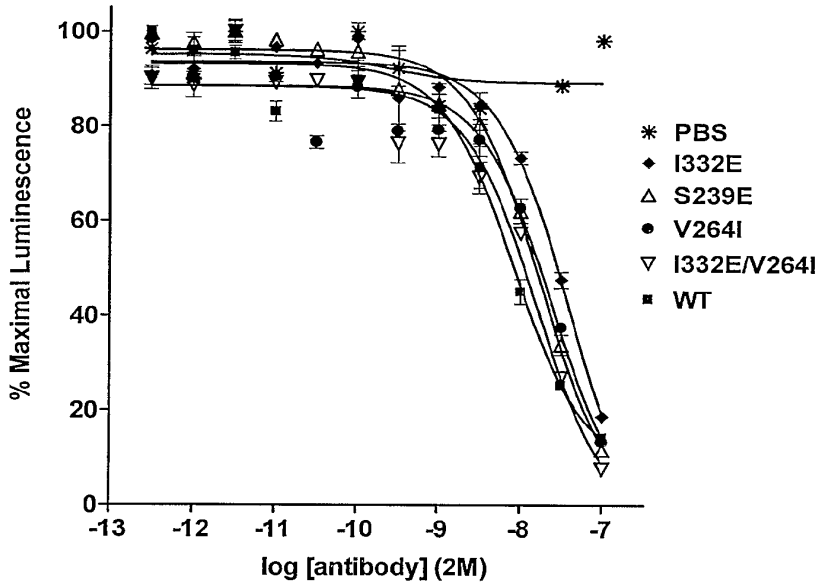

Figure 27b

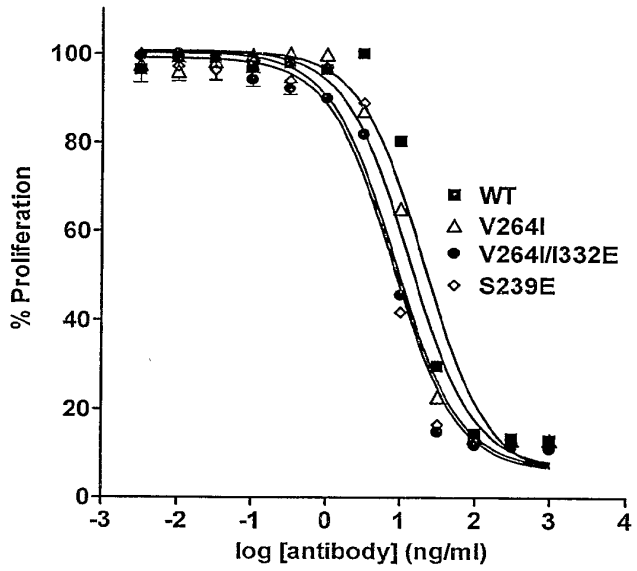

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Figure 28

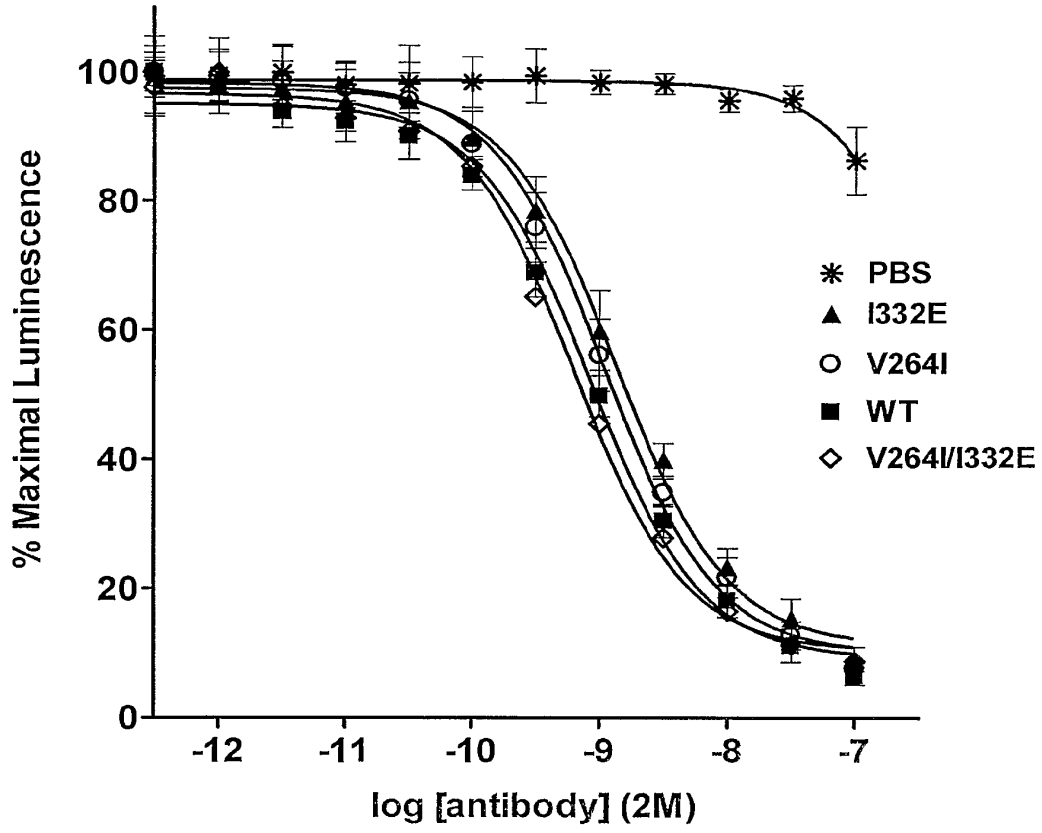
% Maximal Luminescence
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
log [antibody] (2M)
PBS
I332E
V264I
WT
V264I/I332E

Figure 29

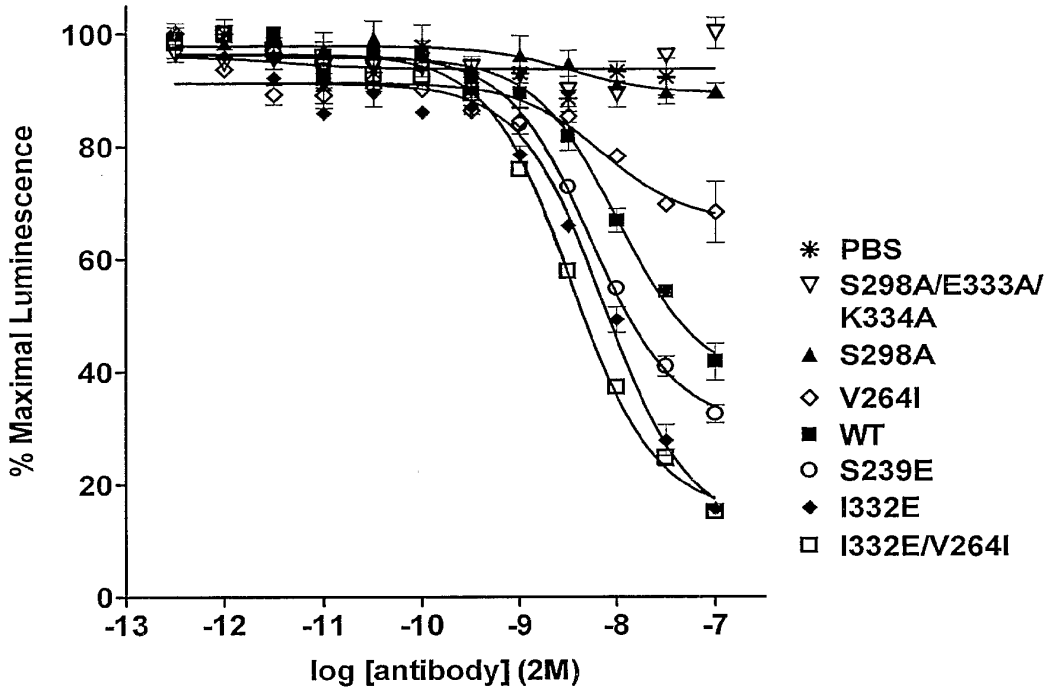

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Figure 30

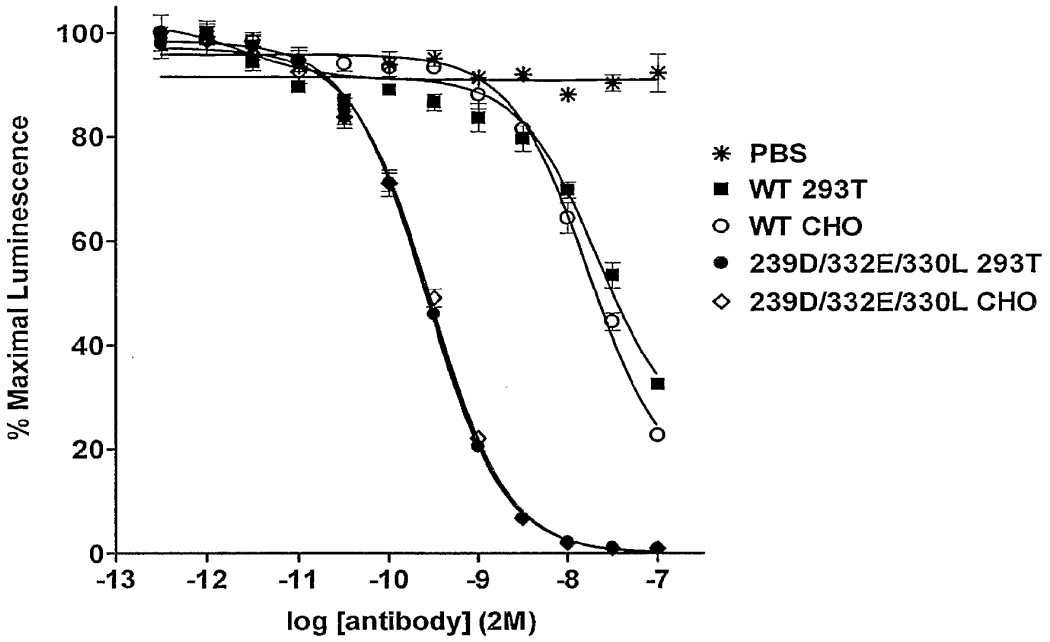

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Figure 31a

Anti-CD20 light chain
QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGSG
TSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS
SPVTKSFNRGEC

Figure 31b

Anti-CD20 heavy chain

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFK
GKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGP**SV**FLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKF**N**WYVDGVEVHNAKTKPREEQY**NS**TYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LP**A**P**I**EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 31c

Anti-CD20 heavy chain comprising possible Fc variants
QVQLQQPGAELVKPGA**S**VKMSCKASGYTFTSY**N**M**H**WVKQTPGRGLEWIGAIYPGNGDTSYNQKFK
GKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFP
LAPSSKSTSGGTA**A**LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGP**X_1X_2**FLFPPKPKDTLMISRTPEVTC
VV**X_3**DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY**X_4Z_1**TYRVVSVLTVLHQDWLNGKEYKCKVSN
KALP**X_5PX_6**EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Position	WT	Possible Substitutions
X_1	S	D, E, N, Q, T
X_2	V	I, M
X_3	V	I, T, Y
X_4	N	D
X_5	A	Y, L, I
X_6	I	D, E, N, Q
Z_1	S	A

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(71) Applicant *(for all designated States except US)*: **ALEXION PHARMACEUTICALS, INC.** [US/US]; 352 Knotter Drive, Cheshire, CT 06410 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BELL, Leonard** [US/US]; 59 Tumblebrook Road, Woodbridge, CT 06525 (US). **ROTHER, Russell, P.** [US/US]; 25 Blue Trail Drive, Prospect, CT 06712 (US).

(74) Agents: **DELUCA, Peter** et al.; Carter, DeLuca, Farrell & Schmidt, LLP, 445 Broadhollow Road, Suite 225, Melville, NY 11747-3615 (US).

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(54) Title: METHOD OF TREATING HEMOLYTIC DISEASE

Biochemical Parameters of Hemolysis During Eculizumab Treatment

Biochemical Marker	Normal range	Time of Analysis			p-value[a]
		Pre-study[b]	12 weeks	64 weeks	
LDH (IU/L)	150 - 480	3110.7 +/- 598.4	594.0 +/- 31.7	622.4 +/-41.1	0.002
AST (IU/L)	10 - 40	76.2 +/- 16.0	26.2 +/- 2.3	30.1 +/- 3.2	0.02
Haptoglobin (g/L)	0.5 - 2	<0.06	<0.06	0.14 +/- 0.07[c]	N.S.[d]
Hemoglobin (g/dL)	11.5 - 18	10.0 +/- 0.4	10.3 +/- 0.4	10.4 +/- 0.4	N.S.
Bilirubin	3 - 15	25.9 +/- 4.3	28.2 +/- 4.4	28.7 +/- 4.0	N.S.
Reticulocytes ($x10^3/mm^3$)	20 - 80	161.4 +/- 25.9	191.2 +/- 23.6	189.6 +/- 21.8	N.S.

[a]from comparisons of mean change from pre-study to 64 weeks
[b]values represent means during 52 week period prior to treatment except for AST which represents the baseline mean
[c]10 of 11 patients were below the detectable limit of haptoglobin (<0.06 g/L); 1 patient had a value of 0.69 g/L.
[d]not significant

(57) Abstract: Paroxysmal nocturnal hemoglobinuria or other hemolytic diseases are treating using a compound which binds to or otherwise blocks the generation and/or the activity of one or more complement components, such as, for example, a complement-inhibiting antibody.

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METHOD OF TREATING HEMOLYTIC DISEASE

RELATED APPLICATIONS

This application claims priority to, and is a continuation-in-part of, U.S. Patent Application Serial No. 10/771,552, filed February 3, 2004, the entire disclosure of which is incorporated herein by this reference.

BACKGROUND

Technical Field

This disclosure relates to a method of treating a hemolytic disease such as, for example, paroxysmal nocturnal hemoglobinuria ("PNH"), by administering a compound which binds to, or otherwise blocks, the generation and/or activity of one or more complement components.

Background of Related Art

Paroxysmal nocturnal hemoglobinuria ("PNH") is an uncommon blood disorder wherein red blood cells are compromised and are thus destroyed more rapidly than normal red blood cells. PNH results from a mutation of bone marrow cells resulting in the generation of abnormal blood cells. More specifically, PNH is believed to be a disorder of hematopoietic stem cells, which give rise to distinct populations of mature blood cells. The basis of the disease appears to be somatic mutations leading to the inability to synthesize the glycosyl-phosphatidylinositol ("GPI") anchor that is responsible for binding

proteins to cell membranes. The mutated gene, PIG-A (phosphatidylinositol glycan class A) resides in the X chromosome and can have several different mutations, varying from deletions to point mutations.

PNH causes a sensitivity to complement proteins and this sensitivity occurs in the cell membrane. PNH cells are deficient in a number of proteins, particularly essential complement-regulating surface proteins. These complement-regulating surface proteins include the decay-accelerating factor ("DAF") or CD55 and membrane inhibitor of reactive lysis ("MIRL") or CD59.

PNH is characterized by hemolytic anemia (a decreased number of red blood cells), hemoglobinuria (the presence of hemoglobin in the urine particularly evident after sleeping), and hemoglobinemia (the presence of hemoglobin in the bloodstream). PNH-afflicted individuals are known to have paroxysms, which are defined here as incidences of dark-colored urine. Hemolytic anemia is due to intravascular destruction of red blood cells by complement components. Other known symptoms include dysphagia, fatigue, erectile dysfunction, thrombosis and recurrent abdominal pain.

Hemolysis resulting from hemolytic diseases causes local and systemic nitric oxide (NO) deficiency through the release of free hemoglobin. Free hemoglobin is a very efficient scavenger of NO, due in part to the accessibility of NO in the non-erythrocyte compartment and a 10^6 times greater affinity of the heme moiety for NO than that for oxygen. The occurrence of intravascular hemolysis often generates sufficient free hemoglobin to completely deplete haptoglobin. Once the capacity of this hemoglobin scavenging protein is exceeded, consumption of endogenous NO ensues. For example, in a setting of intravascular hemolysis such as PNH, where LDH levels can easily exceed

2 - 3 times their normal levels, free hemoglobin would likely obtain concentrations of 0.8 – 1.6 g/l. Since haptoglobin can only bind somewhere between 0.7 to 1.5 g/l of hemoglobin depending on the haptoglobin allotype, a large excess of free hemoglobin would be generated. Once the capacity of hemoglobin reabsorption by the kidney proximal tubules is exceeded, hemoglobinuria ensues. The release of free hemoglobin during intravascular hemolysis results in excessive consumption of NO with subsequent enhanced smooth muscle contraction, vasoconstriction and platelet activation and aggregation. PNH-related morbidities associated with NO scavenging by hemoglobin include abdominal pain, erectile dysfunction, esophageal spasm, and thrombosis.

The laboratory evaluation of hemolysis normally includes hematologic, serologic, and urine tests. Hematologic tests include an examination of the blood smear for morphologic abnormalities of RBCs (to determine causation), and the measurement of the reticulocyte count in whole blood (to determine bone marrow compensation for RBC loss). Serologic tests include lactate dehydrogenase (LDH; widely performed), and free hemoglobin (not widely performed) as a direct measure of hemolysis. LDH levels, in the absence of tissue damage in other organs, can be useful in the diagnosis and monitoring of patients with hemolysis. Other serologic tests include bilirubin or haptoglobin, as measures of breakdown products or scavenging reserve, respectively. Urine tests include bilirubin, hemosiderin, and free hemoglobin, and are generally used to measure gross severity of hemolysis and for differentiation of intravascular vs. extravascular etiologies of hemolysis rather than routine monitoring of hemolysis. Further, RBC numbers, RBC (i.e. cell-

bound) hemoglobin, and hematocrit are generally performed to determine the extent of any accompanying anemia rather than as a measure of hemolytic activity per se.

Steroids have been employed as a therapy for hemolytic diseases and may be effective in suppressing hemolysis in some patients, although long term use of steroid therapy carries many negative side effects. Afflicted patients may require blood transfusions, which carry risks of infection. Anti-coagulation therapy may also be required to prevent blood clot formation. Bone marrow transplantation has been known to cure PNH, however, bone marrow matches are often very difficult to find and mortality rates are high with such procedure.

It would be advantageous to provide a treatment which safely and reliably eliminates and/or limits hemolytic diseases, such as PNH, and their effects.

Summary

Paroxysmal nocturnal hemoglobinuria ("PNH") and other hemolytic diseases are treated in accordance with this disclosure using a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components. Suitable compounds include, for example, antibodies which bind to or otherwise block the generation and/or activity of one or more complement components, such as, for example, an antibody specific to complement component C5. In particularly useful embodiments, the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb (eculizumab), h5G1.1-scFv (pexelizumab) and other functional

fragments of h5G1.1. It has surprisingly been found that the present methods provide improvements in the PNH subject within 24 hours of administration of the compound. For example, hemolysis is significantly reduced within 24 hours of administration of the compound as indicated by resolution of hemoglobinuria.

The complement-inhibiting compound can be administered prophylactically in individuals known to have a hemolytic disease to prevent, or help prevent the onset of symptoms. Alternatively, the complement-inhibiting compound can be administered as a therapeutic regimen to an individual experiencing symptoms of a hemolytic disease.

In another aspect, a method of increasing the proportion of complement sensitive type III red blood cells and therefore the total red blood cell count in a patient afflicted with a hemolytic disease is contemplated. The method comprises administering a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components to a patient afflicted with a hemolytic disease. By increasing type III red blood cell count, symptoms such as fatigue and anemia also can be alleviated in a patient afflicted with a hemolytic disease.

In yet another aspect, the present disclosure contemplates a method of rendering a subject afflicted with a hemolytic disease less dependent on transfusions or transfusion-independent by administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

It has surprisingly been found that patients can be rendered transfusion-independent in accordance with the present methods. Unexpectedly, transfusion-independence can be maintained in some embodiments for twelve months or more, long beyond the 120 day life cycle of red blood cells. In other embodiments, transfusion-independence can be maintained for two years or more. Treatment for six months or more is required for the evaluation of transfusion independence given the long half life of red blood cells.

In another aspect, the present disclosure contemplates a method of treating a nitric oxide (NO) imbalance in a subject by administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components. By reducing the lysis of red blood cells, the present methods reduce the amount of free hemoglobin in the bloodstream, thereby increasing serum levels of nitric oxide (NO). In particularly useful embodiments, NO homeostasis is restored wherein there is a resolution of symptoms attributable to NO deficiency.

In another aspect, the present disclosure contemplates a method of treating thrombosis in a subject by administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

In another aspect, the present disclosure contemplates a method of treating fatigue in a subject afflicted with a hemolytic disease by administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

In another aspect, the present disclosure contemplates a method of treating erectile dysfunction in a subject afflicted with a hemolytic disease by administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

In another aspect, the present disclosure contemplates a method of treating abdominal pain in a subject afflicted with a hemolytic disease by administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

In yet another aspect, the present disclosure contemplates a method of treating a subject afflicted with a hemolytic disease by administering: 1) one or more compounds known to increase hematopoiesis (for example, either by boosting production, eliminating stem cell destruction or eliminating stem cell

inhibition) in combination with 2) a compound selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components. Suitable compounds known to increase hematopoiesis include, for example, steroids, immunosuppressants (such as, cyclosporin), anti-coagulants (such as, warfarin), folic acid, iron and the like, erythropoietin (EPO) and antithymocyte globulin (ATG), antilymphocyte globulin (ALG), EPO derivatives, and darbepoetin alfa (commercially available as Aranesp® from Amgen, Inc., Thousand Oaks, CA (Aranesp® is a man-made form of EPO produced in Chinese hamster ovary (CHO) cells by recombinant DNA technology)). In particularly useful embodiments, erythropoietin (EPO) (a compound known to increase hematopoiesis), EPO derivatives, or darbepoetin alfa may be administered in combination with an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and other functional fragments of h5G1.1.

In yet another aspect, the present disclosure contemplates a method of treating one or more symptoms of hemolytic diseases in a subject where the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10% before or during treatment, by administering a compound selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components, said compound being administered

alone or in combination with one or more compounds known to increase hematopoiesis, such as EPO, EPO derivatives, or darbepoetin alfa.

In yet another aspect, the present disclosure contemplates a method of treating one or more symptoms of hemolytic diseases in a subject having a platelet count above 40,000 per microliter, by administering a compound selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components, said compound being administered alone or in combination with one or more compounds known to increase hematopoiesis, such as EPO, EPO derivatives, or darbepoetin alfa.

In yet another aspect, the present disclosure contemplates a method of treating one or more symptoms of a hemolytic diseases in a subject having a reticulocyte count above 80 x 10^9 per liter, by administering a compound selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components, said compound being administered alone or in combination with one or more compounds known to increase hematopoiesis, such as EPO, EPO derivatives, or darbepoetin alfa.

Brief Description of the Drawings

Figure 1A reports biochemical parameters of hemolysis measured during treatment of PNH patients with an anti-C5 antibody.

Figure 1B graphically depicts the effect of treatment with an anti-C5 antibody on lactate dehydrogenase (LDH) levels.

Figure 2 shows a urine color scale devised to monitor the incidence of paroxysm of hemoglobinuria in PNH patients.

Figure 3 is a graph of the effects of eculizumab treatments on patient paroxysm rates, as compared to pre-treatment rates.

Figure 4 shows urine samples of PNH patients and measurements of hemoglobinuria, dysphagia, LDH, AST, pharmacokinetics (PK) and pharmacodynamics (PD) reflecting the immediate and positive effects of the present methods on hemolysis, symptoms and pharmacodynamics suitable to completely block complement.

Figure 5 graphically depicts the effect of anti-C5 antibody dosing schedule on hemoglobinuria over time.

Figures 6a and 6b are graphs comparing the number of transfusion units required per patient per month, prior to and during treatment with an anti-C5 antibody: Figure 6a depicts cytopenic patients; and Figure 6b depicts non-cytopenic patients.

Figure 7 shows the management of a thrombocytopenic patient by administering an anti-C5 antibody and erythropoietin (EPO).

Figure 8 graphically depicts the pharmacodynamics of an anti-C5 antibody.

Figure 9 is a chart of the results of European Organization for Research and Treatment of Cancer questionnaires ("EORTC QLC-C30") completed during the anti-C5 therapy regimen addressing quality of life issues.

Figure 10 is a chart depicting the effects of anti-C5 antibody treatments on adverse symptoms associated with PNH.

Detailed Description

The present disclosure relates to a method of treating paroxysmal nocturnal hemoglobinuria ("PNH") and other hemolytic diseases in mammals. Specifically, the methods of treating hemolytic diseases, which are described herein, involve using compounds which bind to or otherwise block the generation and/or activity of one or more complement components. The present methods have been found to provide surprising results. For instance, hemolysis rapidly ceases upon administration of the compound which binds to or otherwise blocks the generation and/or activity of one or more complement components, with hemoglobinuria being significantly reduced after treatment. Also, hemolytic patients can be rendered less dependent on transfusions or transfusion-independent for extended periods (twelve months or more), well beyond the 120 day life cycle of red blood cells. In addition, type III red blood cell count can be increased dramatically in the midst of other mechanisms of red blood cell lysis (non-complement mediated and/or earlier complement component mediated e.g., Cb3). Another example of a surprising result is that symptoms resolved, indicating that NO serum levels were increased enough even in the presence of other mechanisms of red blood cell lysis. These and other results reported herein are unexpected and could not be predicted from prior treatments of hemolytic diseases.

Any compound which binds to or otherwise blocks the generation and/or activity of one or more complement components can be used in the

present methods. A specific class of such compounds which is particularly useful includes antibodies specific to a human complement component, especially anti-C5 antibodies. The anti-C5 antibody inhibits the complement cascade and, ultimately, prevents red blood cell ("RBC") lysis by the complement protein complex C5b-9. By inhibiting and/or reducing the lysis of RBCs, the effects of PNH and other hemolytic diseases (including symptoms such as hemoglobinuria, anemia, hemoglobinemia, dysphagia, fatigue, erectile dysfunction, recurrent abdominal pain and thrombosis) are eliminated or decreased.

In another embodiment, soluble forms of the proteins CD55 and CD59, singularly or in combination with each other, can be administered to a subject to inhibit the complement cascade in its alternative pathway. CD55 inhibits at the level of C3, thereby preventing the further progression of the cascade. CD59 inhibits the C5b-8 complex from combining with C9 to form the membrane attack complex (see discussion below).

The complement system acts in conjunction with other immunological systems of the body to defend against intrusion of bacterial and viral pathogens. There are at least 25 proteins involved in the complement cascade, which are found as a complex collection of plasma proteins and membrane cofactors. Complement components achieve their immune defensive functions by interacting in a series of intricate but precise enzymatic cleavage and membrane binding events. The resulting complement cascade leads to the production of products with opsonic, immunoregulatory, and lytic functions. A concise summary of the biologic activities associated with

complement activation is provided, for example, in The Merck Manual, 16th Edition.

The complement cascade progresses via the classical pathway, the alternative pathway or the lectin pathway. These pathways share many components, and while they differ in their initial steps, they converge and share the same "terminal complement" components (C5 through C9) responsible for the activation and destruction of target cells. The classical complement pathway is typically initiated by antibody recognition of and binding to an antigenic site on a target cell. The alternative pathway is usually antibody independent, and can be initiated by certain molecules on pathogen surfaces. Additionally, the lectin pathway is typically initiated with binding of mannose-binding lectin ("MBL") to high mannose substrates. These pathways converge at the point where complement component C3 is cleaved by an active protease to yield C3a and C3b.

C3a is an anaphylatoxin (see discussion below). C3b binds to bacteria and other cells, as well as to certain viruses and immune complexes, and tags them for removal from the circulation. (C3b in this role is known as opsonin.) The opsonic function of C3b is generally considered to be the most important anti-infective action of the complement system. Patients with genetic lesions that block C3b function are prone to infection by a broad variety of pathogenic organisms, while patients with lesions later in the complement cascade sequence, i.e., patients with lesions that block C5 functions, are found to be more prone only to Neisseria infection, and then only somewhat more prone (Fearon, in Intensive Review of Internal Medicine, 2nd Ed. Fanta and Minaker, eds. Brigham and Women's and Beth Israel Hospitals, 1983).

C3b also forms a complex with other components unique to each pathway to form classical or alternative C5 convertase, which cleaves C5 into C5a and C5b. C3 is thus regarded as the central protein in the complement reaction sequence since it is essential to all three activation pathways (Wurzner, et al., Complement Inflamm. 8:328-340, 1991). This property of C3b is regulated by the serum protease Factor I, which acts on C3b to produce iC3b (inactive C3b). While still functional as an opsonin, iC3b can not form an active C5 convertase.

The pro-C5 precursor is cleaved after amino acid 655 and 659, to yield the beta chain as an amino terminal fragment (amino acid residues +1 to 655 of the sequence) and the alpha chain as a carboxyl terminal fragment (amino acid residues 660 to 1658 of the sequence), with four amino acids (amino acid residues 656-659 of the sequence) deleted between the two. C5 is glycosylated, with about 1.5-3 percent of its mass attributed to carbohydrate. Mature C5 is a heterodimer of a 999 amino acid 115 kDa alpha chain that is disulfide linked to a 655 amino acid 75 kDa beta chain. C5 is found in normal serum at approximately 75 µg/ml (0.4 µM). C5 is synthesized as a single chain precursor protein product of a single copy gene (Haviland et al. J. Immunol. 1991, 146:362-368). The cDNA sequence of the transcript of this gene predicts a secreted pro-C5 precursor of 1658 amino acids along with an 18 amino acid leader sequence (see, U.S. patent 6,355,245).

Cleavage of C5 releases C5a, a potent anaphylatoxin and chemotactic factor, and leads to the formation of the lytic terminal complement complex, C5b-9. C5a is cleaved from the alpha chain of C5 by either alternative or classical C5 convertase as an amino terminal fragment comprising the first 74

amino acids of the alpha chain (i.e., amino acid residues 660-733 of the sequence). Approximately 20 percent of the 11 kDa mass of C5a is attributed to carbohydrate. The cleavage site for convertase action is at, or immediately adjacent to, amino acid residue 733 of the sequence. A compound that binds at, or adjacent, to this cleavage site would have the potential to block access of the C5 convertase enzymes to the cleavage site and thereby act as a complement inhibitor.

C5b combines with C6, C7, and C8 to form the C5b-8 complex at the surface of the target cell. Upon binding of several C9 molecules, the membrane attack complex ("MAC", C5b-9, terminal complement complex--TCC) is formed. When sufficient numbers of MACs insert into target cell membranes, the openings they create (MAC pores) mediate rapid osmotic lysis of the target cells. Lower, non-lytic concentrations of MACs can produce other proinflammatory effects. In particular, membrane insertion of small numbers of the C5b-9 complexes into endothelial cells and platelets can cause deleterious cell activation. In some cases activation may precede cell lysis.

C5a and C5b-9 also have pleiotropic cell activating properties, by amplifying the release of downstream inflammatory factors, such as hydrolytic enzymes, reactive oxygen species, arachidonic acid metabolites and various cytokines. C5 can also be activated by means other than C5 convertase activity. Limited trypsin digestion (Minta and Man, J. Immunol. 1977, 119:1597-1602; Wetsel and Kolb, J. Immunol. 1982, 128:2209-2216) and acid treatment (Yammamoto and Gewurz, J. Immunol. 1978, 120:2008; Damerau

et al., Molec. Immunol. 1989, 26:1133-1142) can also cleave C5 and produce active C5b.

As mentioned above, C3a and C5a are anaphylatoxins. These activated complement components can trigger mast cell degranulation, which releases histamine and other mediators of inflammation, resulting in smooth muscle contraction, increased vascular permeability, leukocyte activation, and other inflammatory phenomena including cellular proliferation resulting in hypercellularity. C5a also functions as a chemotactic peptide that serves to attract pro-inflammatory granulocytes to the site of complement activation.

Any compounds which bind to or otherwise block the generation and/or activity of any of the human complement components may be utilized in accordance with the present disclosure. In some embodiments, antibodies specific to a human complement component are useful herein. Some compounds include antibodies directed against complement components C-1, C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2, thus preventing the generation of the anaphylatoxic activity associated with C5a and/or preventing the assembly of the membrane attack complex associated with C5b.

Also useful in the present methods are naturally occurring or soluble forms of complement inhibitory compounds such as CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH. Other compounds which may be utilized to bind to or otherwise block the generation and/or activity of any of the human complement components include, but are not limited to, proteins, protein fragments, peptides, small molecules, RNA aptamers including ARC187 (which is commercially available

from Archemix Corp., Cambridge, MA), L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, molecules which may be utilized in RNA interference (RNAi) such as double stranded RNA including small interfering RNA (siRNA), locked nucleic acid (LNA) inhibitors, peptide nucleic acid (PNA) inhibitors, etc.

Functionally, one suitable class of compounds inhibits the cleavage of C5, which blocks the generation of potent proinflammatory molecules C5a and C5b-9 (terminal complement complex). Preferably, the compound does not prevent the formation of C3b, which subserves critical immunoprotective functions of opsonization and immune complex clearance.

While preventing the generation of these membrane attack complex molecules, inhibition of the complement cascade at C5 preserves the ability to generate C3b, which is critical for opsonization of many pathogenic microorganisms, as well as for immune complex solubilization and clearance. Retaining the capacity to generate C3b appears to be particularly important as a therapeutic factor in complement inhibition for hemolytic diseases, where increased susceptibility to thrombosis, infection, fatigue, lethargy and impaired clearance of immune complexes are pre-existing clinical features of the disease process.

Particularly useful compounds for use herein are antibodies that reduce, directly or indirectly, the conversion of complement component C5 into complement components C5a and C5b. One class of useful antibodies are those having at least one antigen binding site and exhibiting specific binding to human complement component C5. Particularly useful complement inhibitors are compounds which reduce the generation of C5a

and/or C5b-9 by greater than about 30%. Anti-C5 antibodies that have the desirable ability to block the generation of C5a have been known in the art since at least 1982 (Moongkarndi et al. Immunobiol. 1982, 162:397; Moongkarndi et al. Immunobiol. 1983, 165:323). Antibodies known in the art that are immunoreactive against C5 or C5 fragments include antibodies against the C5 beta chain (Moongkarndi et al. Immunobiol. 1982, 162:397; Moongkarndi et al. Immunobiol. 1983, 165:323; Wurzner et al. 1991, supra; Mollnes et al. Scand. J. Immunol. 1988, 28:307-312); C5a (see for example, Ames et al. J. Immunol. 1994, 152:4572-4581, U.S. Pat. No. 4,686,100, and European patent publication No. 0 411 306); and antibodies against non-human C5 (see for example, Giclas et al. J. Immunol. Meth. 1987, 105:201-209). Particularly useful anti-C5 antibodies are h5G1.1-mAb, h5G1.1-scFv and other functional fragments of h5G1.1. Methods for the preparation of h5G1.1-mAb, h5G1.1-scFv and other functional fragments of h5G1.1 are described in U.S. Patent No. 6,355,245 and "Inhibition of Complement Activity by Humanized Anti-C5 Antibody and Single Chain Fv", Thomas et al., *Molecular Immunology*, Vol. 33, No. 17/18, pages 1389-1401, 1996, the disclosures of which are incorporated herein in their entirety by this reference. The antibody h5G1.1-mAb is currently undergoing clinical trials under the tradename eculizumab.

Hybridomas producing monoclonal antibodies reactive with complement component C5 can be obtained according to the teachings of Sims, et al., U.S. Pat. No. 5,135,916. Antibodies are prepared using purified components of the complement C5 component as immunogens according to known methods. In accordance with this disclosure, complement component

C5, C5a or C5b is preferably used as the immunogen. In accordance with particularly preferred useful embodiments, the immunogen is the alpha chain of C5.

Particularly useful antibodies share the required functional properties discussed in the preceding paragraph and have any of the following characteristics:

(1) they compete for binding to portions of C5 that are specifically immunoreactive with 5G1.1;

(2) they specifically bind to the C5 alpha chain -- such specific binding, and competition for binding can be determined by various methods well known in the art, including the plasmon surface resonance method (Johne et al., J. Immunol. Meth. 1993, 160:191-198); and

(3) they block the binding of C5 to either C3 or C4 (which are components of the C5 convertases).

The compound that inhibits the production and/or activity of at least one complement component can be administered in a variety of unit dosage forms. The dose will vary according to the particular compound employed. For example, different antibodies may have different masses and/or affinities, and thus require different dosage levels. Antibodies prepared as fragments (e.g., Fab, $F(ab')_2$, scFv) will also require differing dosages than the equivalent intact immunoglobulins, as they are of considerably smaller mass than intact immunoglobulins, and thus require lower dosages to reach the same molar levels in the patient's blood.

The dose will also vary depending on the manner of administration, the particular symptoms of the patient being treated, the overall health, condition, size, and age of the patient, and the judgment of the prescribing physician.

Administration of the compound that inhibits the production and/or activity of at least one complement component will generally be in an aerosol form with a suitable pharmaceutical carrier, via intravenous infusion by injection, subcutaneous injection, orally, or sublingually. Other routes of administration may be used if desired.

It is further contemplated that a combination therapy can be used wherein a complement-inhibiting compound is administered in combination with a regimen of known therapy for hemolytic disease. Such regimens include administration of 1) one or more compounds known to increase hematopoiesis (for example, either by boosting production, eliminating stem cell destruction or eliminating stem cell inhibition) in combination with 2) a compound selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components. Suitable compounds known to increase hematopoiesis include, for example, steroids, immunosuppressants (such as, cyclosporin), anti-coagulants (such as, warfarin), folic acid, iron and the like, erythropoietin (EPO) and antithymocyte globulin (ATG), antilymphocyte globulin (ALG), EPO derivatives, and darbepoetin alfa (commercially available as Aranesp® from Amgen, Inc., Thousand Oaks, CA (Aranesp® is a man-made form of EPO produced in Chinese hamster ovary (CHO) cells by recombinant DNA technology)). In

particularly useful embodiments, erythropoietin (EPO) (a compound known to increase hematopoiesis), EPO derivatives, or darbepoetin alfa may be administered in combination with an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and other functional fragments of h5G1.1.

Formulations suitable for injection are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed. (1985). Such formulations must be sterile and non-pyrogenic, and generally will include a pharmaceutically effective carrier, such as saline, buffered (e.g., phosphate buffered) saline, Hank's solution, Ringer's solution, dextrose/saline, glucose solutions, and the like. The formulations may contain pharmaceutically acceptable auxiliary substances as required, such as, tonicity adjusting agents, wetting agents, bactericidal agents, preservatives, stabilizers, and the like.

The present disclosure contemplates methods of reducing hemolysis in a patient afflicted with a hemolytic disease by administering one or more compounds which bind to or otherwise block the generation and/or activity of one or more complement components. Reducing hemolysis means that the duration of time a person suffers from hemolysis is reduced by about 25% or more. The effectiveness of the treatment can be evaluated in any of the various manners known to those skilled in the art for determining the level of hemolysis in a patient. One qualitative method for detecting hemolysis is to observe the occurrences of hemoglobinuria. Quite surprisingly, treatment in accordance with the present methods reduces hemolysis as determined by a rapid reduction in hemoglobinuria.

A more qualitative manner of measuring hemolysis is to measure lactate dehydrogenase (LDH) levels in the patient's bloodstream. LDH catalyzes the interconversion of pyruvate and lactate. Red blood cells metabolize glucose to lactate, which is released into the blood and is taken up by the liver. LDH levels are used as an objective indicator of hemolysis. As those skilled in the art will appreciate, measurements of "upper limit of normal" levels of LDH will vary from lab to lab depending on a number of factors including the particular assay employed and the precise manner in which the assay is conducted. Generally speaking, however, the present methods can reduce hemolysis in a patient afflicted with a hemolytic disease as reflected by a reduction of LDH levels in the patients to within 20% of the upper limit of normal LDH levels. Alternatively, the present methods can reduce hemolysis in a patient afflicted with a hemolytic disease as reflected by a reduction of LDH levels in the patients of greater than 50% of the patient's pre-treatment LDH level, preferably greater than 65% of the patient's pre-treatment LDH level, most preferably greater than 80% of the patient's pre-treatment LDH level.

Another quantitative measurement of a reduction in hemolysis is the presence of GPI-deficient red blood cells (Type III red blood cells). As those skilled in the art will appreciate, Type III red blood cells have no GPI-anchor protein expression on the cell surface. The proportion of GPI-deficient cells can be determined by flow cytometry using, for example, the technique described in Richards, et al., *Clin. Appl. Immunol. Rev.*, vol. 1, pages 315-330, 2001. The present methods can reduce hemolysis in a patient afflicted with a hemolytic disease as reflected by an increase in Type III red blood

cells. Preferably an increase in Type III red blood cell levels in the patient of greater than 25% of the total red blood cell count is achieved, more preferably an increase in Type III red blood cell levels in the patients greater than 50% of the total red blood cell count is achieved, most preferably an increase in Type III red blood cell levels in the patients greater than 75% of the total red blood cell count is achieved.

Methods of reducing one or more symptoms associated with PNH or other hemolytic diseases are also within the scope of the present disclosure. Such symptoms include, for example, abdominal pain, fatigue, dyspnea and insomnia. Symptoms can be the direct result of lysis of red blood cells (e.g., hemoglobinuria, anemia, fatigue, low red blood cell count, etc.) or the symptoms can result from low nitric oxide (NO) levels in the patient's bloodstream (e.g., abdominal pain, erectile dysfunction, dysphagia, thrombosis, etc.). It has recently been reported that almost all patients with greater than 40% PNH type III granulocyte clone have thrombosis, abdominal pain, erectile dysfunction and dysphagia, indicating a high hemolytic rate (see Moyo et al., British J. Haematol. 126:133-138 (2004)).

In particularly useful embodiments, the present methods provide a reduction in one or more symptoms associated with PNH or other hemolytic diseases in a patient having a platelet count in excess of 40,000 per microliter (a hypoplastic patient), preferably in excess of 75,000 per microliter, most preferably in excess of 150,000 per microliter. In other embodiments, the present methods provide a reduction in one or more symptoms associated with PNH or other hemolytic diseases in a patient where the proportion of PNH type III red blood cells of the subject's total red blood cell content is

greater than 10%, preferably greater than 25%, most preferably in excess of 50%. In yet other embodiments, the present methods provide a reduction in one or more symptoms associated with PNH or other hemolytic diseases in a patient having a reticulocyte count in excess of 80 x 10^9 per liter, more preferably in excess of 120 x 10^9 per liter, most preferably in excess of 150 x 10^9 per liter. Patients in the most preferable ranges recited above have active bone marrow and will produce adequate numbers of red blood cells. While in a patient afflicted with PNH or other hemolytic disease the red blood cells may be defective in one or more ways (e.g., GPI deficient), the present methods are particularly useful in protecting such cells from lysis resulting from complement activation. Thus, patients within the preferred ranges benefit most from the present methods.

In one aspect, a method of reducing fatigue is contemplated, the method including the step of administering to a subject having or susceptible to a hemolytic disease a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components. Reducing fatigue means the duration of time a person suffers from fatigue is reduced by about 25% or more. Fatigue is a symptom believed to be associated with intravascular hemolysis as the fatigue relents when hemoglobinuria resolves even in the presence of anemia. By reducing the lysis of red blood cells, the present methods reduce fatigue. Patients within the above-mentioned preferred ranges of type III red blood cells, reticulocytes and platelets benefit most from the present methods.

In another aspect, a method of reducing abdominal pain is contemplated, the method including the step of administering to a subject

having or susceptible to a hemolytic disease a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components. Reducing abdominal pain means the duration of time a person suffers from abdominal pain is reduced by about 25% or more. Abdominal pain is a symptom resulting from the inability of a patient's natural levels of haptoglobin to process all the free hemoglobin released into the bloodstream as a result of intravascular hemolysis, resulting in the scavenging of NO and intestinal dystonia and spasms. By reducing the lysis of red blood cells, the present methods reduce the amount of free hemoglobin in the bloodstream, thereby reducing abdominal pain. Patients within the above-mentioned preferred ranges of type III red blood cells, reticulocytes and platelets benefit most from the present methods.

In another aspect, a method of reducing dysphagia is contemplated, the method including the step of administering to a subject having or susceptible to a hemolytic disease a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components. Reducing dysphagia means the duration of time a person has dysphagia attacks is reduced by about 25% or more. Dysphagia is a symptom resulting from the inability of a patient's natural levels of haptoglobin to process all the free hemoglobin released into the bloodstream as a result of intravascular hemolysis, resulting in the scavenging of NO and esophageal spasms. By reducing the lysis of red blood cells, the present methods reduce the amount of free hemoglobin in the bloodstream, thereby reducing dysphagia. Patients within the above-mentioned preferred ranges of type III red blood cells, reticulocytes and platelets benefit most from the present methods.

In yet another aspect, a method of reducing erectile dysfunction is contemplated, the method including the step of administering to a subject having or susceptible to a hemolytic disease a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components. Reducing erectile dysfunction means the duration of time a person suffers from erectile dysfunction is reduced by about 25% or more. Erectile dysfunction is a symptom believed to be associated with scavenging of NO by free hemoglobin released into the bloodstream as a result of intravascular hemolysis. By reducing the lysis of red blood cells, the present methods reduce the amount of free hemoglobin in the bloodstream, thereby increasing serum levels of NO and reducing erectile dysfunction. Patients within the above-mentioned preferred ranges of type III red blood cells, reticulocytes and platelets benefit most from the present methods.

In yet another aspect, a method of reducing hemoglobinuria is contemplated, the method including the step of administering to a subject having or susceptible to a hemolytic disease a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components. Reducing hemoglobinuria means a reduction in the number of times a person has red, brown, or darker urine, wherein the reduction is typically about 25% or more. Hemoglobinuria is a symptom resulting from the inability of a patient's natural levels of haptoglobin to process all the free hemoglobin released into the bloodstream as a result of intravascular hemolysis. By reducing the lysis of red blood cells, the present methods reduce the amount of free hemoglobin in the bloodstream and urine thereby reducing hemoglobinuria. Quite surprisingly, the reduction in hemoglobinuria

occurs rapidly. Patients within the above-mentioned preferred ranges of type III red blood cells, reticulocytes and platelets benefit most from the present methods.

In still another aspect, a method of reducing thrombosis is contemplated, the method including the step of administering to a subject having or susceptible to a hemolytic disease a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components. Reducing thrombosis means the duration of time a person has thrombosis attacks is reduced by about 25% or more. Thrombosis is a symptom believed to be associated with scavenging of NO by free hemoglobin released into the bloodstream as a result of intravascular hemolysis and/or the lack of CD59 on the surface of platelets resulting in terminal complement mediated activation of the platelet. By reducing the lysis of red blood cells, the present methods reduce the amount of free hemoglobin in the bloodstream, thereby increasing serum levels of NO and reducing thrombosis. In addition, blockade of complement will prevent terminal complement-mediated activation of platelets and thrombosis. C5a will also be inhibited by this method which can induce platelet aggregation through C5a receptors on platelets and endothelial cells.

Thrombosis is thought to be multi-factorial in etiology including NO scavenging by free hemoglobin, the absence of terminal complement inhibition on the surface of circulating platelets and changes in the endothelium surface by cell free heme. The intravascular release of free hemoglobin may directly contribute to small vessel thrombosis. NO has been shown to inhibit platelet aggregation, induce disaggregation of aggregated

platelets and inhibit platelet adhesion. Conversely, NO scavenging by hemoglobin or the reduction of NO generation by the inhibition of arginine metabolism results in an increase in platelet aggregation. PNH platelets also lack the terminal complement inhibitor CD59 and multiple studies have shown that deposition of terminal complement (C5b-9) on platelets causes membrane vesiculation and the generation of microvesicles. The microvesicles act as a site for the generation of the clotting components factor Va, Xa or the prothrombinase complex. It is thought that these particles may also contribute to the genesis of thrombosis in PNH. By reducing the lysis of red blood cells, the present methods reduce the amount of free hemoglobin in the bloodstream, thereby increasing serum levels of NO and reducing thrombosis. In addition, inhibiting complement at C5 will prevent C5b-9 and C5a mediated activation of platelets and/or endothelial cells.

In particularly useful embodiments, the present methods reduce thrombosis, especially patients having a platelet count in excess of 40,000 per microliter, preferably in excess of 75,000 per microliter, most preferably in excess of 150,000 per microliter. In other embodiments, the present methods reduce thrombosis in patients where the proportion of PNH type III red blood cells of the subject's total red blood cell content is greater than 10%, preferably greater than 25%, more preferably in excess of 50%, most preferably in excess of 75%. In yet other embodiments, the present methods reduce transfusion thrombosis in patients having a reticulocyte count in excess of 80 x 10^9 per liter, more preferably in excess of 120 x 10^9 per liter, most preferably in excess of 150 x 10^9 per liter.

In still another aspect, a method of reducing anemia is contemplated, the method including the step of administering to a subject having or susceptible to a hemolytic disease a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components. Reducing anemia means the duration of time a person has anemia is reduced by about 25% or more. Anemia in hemolytic diseases results from the blood's reduced capacity to carry oxygen due to the loss of red blood cell mass. By reducing the lysis of red blood cells, the present methods assist red blood cell levels to increase thereby reducing anemia.

In another aspect, a method of increasing the proportion of complement sensitive type III red blood cells and therefore the total red blood cell count in a patient afflicted with a hemolytic disease is contemplated. By increasing the patient's RBC count, fatigue, anemia and the patient's need for blood transfusions is reduced. The reduction in transfusions can be in frequency of transfusions, amount of blood units transfused, or both.

The method of increasing red blood cell count in a patient afflicted with a hemolytic disease includes the step of administering a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components to a patient afflicted with a hemolytic disease. In particularly useful embodiments, the present methods increase red blood cell count in a patient afflicted with a hemolytic disease, especially patients having a platelet count in excess of 40,000 per microliter, preferably in excess of 75,000 per microliter, most preferably in excess of 150,000 per microliter. In other embodiments, the present methods increase red blood cell count in a patient afflicted with a hemolytic disease where the proportion of PNH type III

red blood cells of the subject's total red blood cell content is greater than 10%, preferably greater than 25%, more preferably in excess of 50%, most preferably in excess of 75%. In yet other embodiments, the present methods increase red blood cell count in a patient afflicted with a hemolytic disease having a reticulocyte count in excess of 80 x 10^9 per liter, more preferably in excess of 120 x 10^9 per liter, most preferably in excess of 150 x 10^9 per liter. In some embodiments, the methods of the present disclosure may result in a decrease in the frequency of transfusions by about 50%, typically a decrease in the frequency of transfusions by about 70%, more typically a decrease in the frequency of transfusions by about 90%.

In yet another aspect, the present disclosure contemplates a method of rendering a subject afflicted with a hemolytic disease less dependent on transfusions or transfusion-independent by administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components. As those skilled in the art will appreciate, the normal life cycle for a red blood cell is about 120 days. Treatment for six months or more is required for the evaluation of transfusion independence given the long half life of red blood cells. It has unexpectedly been found that in some patients transfusion-independence can be maintained for twelve months or more, in some cases more than two years, long beyond the 120 day life cycle of red blood cells. In particularly useful embodiments, the present methods provide decreased dependence on transfusions or transfusion-independence in a patient afflicted

with a hemolytic disease, especially patients having a platelet count in excess of 40,000 per microliter, preferably in excess of 75,000 per microliter, most preferably in excess of 150,000 per microliter. In other embodiments, the present methods provide decreased dependence on transfusions or transfusion-independence in a patient afflicted with a hemolytic disease where the proportion of PNH type III red blood cells of the subject's total red blood cell content is greater than 10%, preferably greater than 25%, more preferably in excess of 50%, most preferably in excess of 75%. In yet other embodiments, the present methods provide decreased dependence on transfusions or transfusion-independence in a patient afflicted with a hemolytic disease having a reticulocyte count in excess of 80×10^9 per liter, more preferably in excess of 120×10^9 per liter, most preferably in excess of 150×10^9 per liter.

Methods of increasing the nitric oxide (NO) levels in a patient having PNH or some other hemolytic disease are also within the scope of the present disclosure. These methods of increasing NO levels include the step of administering to a subject having or susceptible to a hemolytic disease a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components. Low NO levels arise in patients afflicted with PNH or other hemolytic diseases as a result of scavenging of NO by free hemoglobin released into the bloodstream as a result of intravascular hemolysis. By reducing the lysis of red blood cells, the present methods reduce the amount of free hemoglobin in the bloodstream, thereby increasing serum levels of NO. In particularly useful embodiments, NO homeostasis is

restored as evidenced by a resolution of symptoms attributable to NO deficiencies.

EXAMPLES

Eleven patients participated in therapy trials to evaluate the effects of anti-C5 antibody on PNH and symptoms associated therewith. PNH patients were transfusion-dependent and hemolytic. Patients were defined as transfusion dependent with a history of four or more transfusions within twelve months. The median number of transfusions within the patient pool was nine in the previous twelve months. The median number of transfusion units used in the previous twelve months was twenty-two for the patient pool.

Over the course of four weeks, each of 11 patients received a weekly 600 mg intravenous infusion of anti-C5 antibody for approximately thirty minutes. The specific anti-C5 antibody used in the study was eculizumab. Patients received 900 mg of eculizumab 1 week later then 900 mg on a bi-weekly basis. The first twelve weeks of the study constituted the pilot study. Following completion of the initial acute phase twelve week study, all patients participated in an extension study conducted to a total of 64 weeks. Ten of the eleven patients participated in an extension study conducted to a total of two years.

The effect of anti-C5 antibody treatments on PNH type III red blood cells ("RBCs") was tested. "PNH Type" refers to the density of GPI-anchored proteins expressed on the cell surface. Type I is normal expression, Type II is intermediate expression, and Type III has no GPI-anchor protein expression on the cell surface. The proportion of GPI-deficient cells is determined by flow

cytometry in the manner described in Richards, et al., *Clin. Appl. Immunol. Rev.*, vol. 1, pages 315-330, 2001. As compared to pre-therapy conditions, PNH Type III red blood cells increased more than 50% during the extension study. The increase from a pre-study mean value of 36.7% of all red blood cells to a 64 week mean value of 58.4% of Type III red blood cells indicated that hemolysis had decreased sharply. See Table 1, below. Eculizumab therapy protected PNH type III RBCs from complement–mediated lysis, prolonging the cells survival. This protection of the PNH–affected cells reduced the need for transfusions, paroxysms and overall hemolysis in all patients in the trial.

Table 1
PNH Cell Populations
Pre- and Post- Eculizumab Treatment in All Patients

	Proportion of PNH Cells (%)			
PNH Cell Type	baseline	12 weeks	64 weeks	p-value[a]
Type III RBCs	36.7 +/- 5.9	59.2 +/- 8.0	58.4 +/- 8.5	0.005
Type II RBCs	5.3 +/- 1.4	7.5 +/- 2.1	13.2 +/- 2.4	0.013
Type III WBCs	92.1 +/- 4.6	89.9 +/- 6.6	91.1 +/- 5.8	N.S.
Type III Platelets	92.4 +/- 2.4	93.3 +/- 2.8	92.8+/- 2.6	N.S.

[a]comparison of mean change from baseline to 64 weeks

During the course of the two year extension study, it was found that PNH red cells with a complete deficiency of GPI-linked proteins (Type III red cells) progressively increased during the treatment period from a mean of 36.7% to 58.9% (p=0.001) while partially deficient PNH red cells (Type II) increased from 5.3% to 8.7% (p=0.01). There was no concomitant change in

the proportion of PNH neutrophils in any of the patients during eculizumab therapy, indicating that the increase in the proportion of PNH red cells was due to a reduction in hemolysis and transfusions rather than a change in the PNH clone(s) themselves.

The effect of anti-C5 antibody treatments on lactate dehydrogenase levels ("LDH") was measured on all eleven patients. LDH catalyzes the interconversion of pyruvate and lactate. Red blood cells metabolize glucose to lactate, which is released into the blood and is taken up by the liver. LDH levels are used as an objective indicator of hemolysis. The LDH levels were decreased by greater than 80% as compared to pre-treatment levels. The LDH levels were lowered from a pre-study mean value of 3111 U/L to a mean value of 594 U/L during the pilot study and a mean value of 622 U/L after 64 weeks ($p = 0.002$ for 64 week comparison; see Figures 1A and 1B).

Similarly, aspartate aminotransferase (AST) levels, another marker of red blood cell hemolysis, decreased from a mean baseline value of 76 IU/L to 26 IU/L and 30 IU/L during the 12 and 64 weeks of treatment, respectively ($p=0.02$ for 64 week comparison). Levels of haptoglobin, hemoglobin and bilirubin, and numbers of reticulocytes, did not change significantly from prestudy values during the 64 week treatment period.

Paroxysm rates were measured and compared to pre-treatment levels. Paroxysm as used in this disclosure is defined as incidences of dark-colored urine with a colorimetric level of 6 of more on a scale of 1-10. Figure 2 shows the urine color scale devised to monitor the incidence of paroxysm of hemoglobinuria in patients with PNH before and during treatment. As compared to pre-treatment levels, the paroxysm percentage rate was reduced

by 93% (see, Figure 3) from 3.0 paroxysms per patient per month before eculizumab treatment to 0.1 paroxysms per patient per month during the initial 12 weeks and 0.2 paroxysms per patient per month during the 64 week treatment (Figure 3 ($p< 0.001$)).

Serum hemolytic activity in nine of the eleven patients was completely blocked throughout the 64 week treatment period with trough levels of eculizumab at equilibrium ranging from approximately 35 µg/ml to 350 µg/ml. During the extension study, 2 patients did not sustain levels of eculizumab necessary to consistently block complement. This breakthrough in serum hemolytic activity occurred in the last 2 days of the 14 day dosing interval, a pattern that was repeated between multiple doses. In one of the patients, as seen in Figure 4, break-through of complement blockade resulted in hemoglobinuria, dysphagia, and increased LDH and AST, which correlated with the return of serum hemolytic activity. At the next dose, symptoms resolved (Figure 5) and reduction in the dosing interval from 900 mg every 14 days to 900 mg every 12 days resulted in a regain of complement control which was maintained over the extension study to 64 weeks in both patients. This patient showed a 24 hour resolution of dysphagia and hemoglobinuria. A reduction in the dosing interval from 14 to 12 days was sufficient to maintain levels of eculizumab above 35 µg/ml and effectively and consistently blocked serum hemolytic activity for the remainder of the extension study for both patients.

The patients' need for transfusions was also reduced by the treatment with eculizumab. Figure 6a compares the number of transfusion units required per patient per month, prior to and during treatment with an anti-C5

antibody for cytopenic patients, while Figure 6b compares the number of transfusion units required per patient per month, prior to and during treatment with an anti-C5 antibody for non-cytopenic patients. A significant reduction in the need for transfusion was also noted in the entire group (mean transfusion rates decreased from 2.1 units per patient per month during a 1 year period prior to treatment to 0.6 units per patient per month during the initial 12 weeks and 0.5 units per patient per month during the combined 64 week treatment period), with non-cytopenic patients benefiting the most. In fact, four of the non-thrombocytopenic patients with normal platelet counts (≥150,000 per microliter) became transfusion-independent during the 64 week treatment.

The effect of eculizumab administered in combination with erythropoietin (EPO) was also evaluated in a thrombocytopenic patient. EPO (NeoRecormon®, Roche Pharmaceuticals, Basel, Switzerland) was administered in an amount of 18,000 I.U. three times per week beginning in week 23 of the study. As shown in Figure 7, the frequency of transfusions required for this patient was significantly reduced, and soon halted.

For the two year extension study, 10 of the 11 patients from the initial 3 month study continued to receive 900 mg of eculizumab every other week. (One patient discontinued eculizumab therapy after 23 months.) Six of the 11 patients had normal platelets (no clinical evidence of marrow failure) whereas 5 of the 11 had low platelets. For the patient who discontinued eculizumab therapy after 23 months, intravascular hemolysis was successfully controlled by eculizumab, but the patient continued to be transfused even after erythropoietin therapy. This patient had the most severe hypoplasia at the start of eculizumab therapy with a platelet count below $30x10^9/l$, suggesting

that the ongoing transfusions were likely a result of the underlying bone marrow failure.

Results of the two year extension study also demonstrated that there was a statistically significant decrease in transfusion requirements for the patients. Three patients remained transfusion independent during the entire two year treatment period, and four cytopenic patients became transfusion independent, three following treatment with EPO (NeoRecormon®). The reduction in transfusion requirements was found to be most pronounced in patients with a good marrow reserve.

Pharmacodynamic levels were measured and recorded according to eculizumab doses. The pharmacodynamic analysis of eculizumab was determined by measuring the capacity of patient serum samples to lyse chicken erythrocytes in a standard total human serum complement hemolytic assay. Briefly, patient samples or human control serum (Quidel, San Diego CA) was diluted to 40% vol/vol with gelatin veronal-buffered saline (GVB2+, Advanced Research technologies, San Diego, CA) and added in triplicate to a 96-well plate such that the final concentrations of serum in each well was 20%. The plate was then incubated at room temperature while chicken erythrocytes (Lampire Biologics, Malvern, PA) were washed. The chicken erythrocytes were sensitized by the addition of anti-chicken red blood cell polyclonal antibody (0.1% vol/vol). The cells were then washed and resuspended in GVB2+ buffer. Chicken erythrocytes (2.5×10^6 cells/30 μL) were added to the plate containing human control serum or patient samples and incubated at 37°C for 30 min. Each plate contained six additional wells of identically prepared chicken erythrocytes of which four wells were incubated

with 20% serum containing 2 mM EDTA as the blank and two wells were incubated with GVB2+ buffer alone as a negative control for spontaneous hemolysis. The plate was then centrifuged and the supernatant transferred to a new flat bottom 96-well plate. Hemoglobin release was determined at OD 415 nm using a microplate reader. The percent hemolysis was determined using the following formula:

$$\text{Percent Hemolysis} = 100 \times \frac{\text{(OD patient sample-OD blank)}}{\text{(OD human serum control-OD blank)}}$$

The graph of the pharmacodynamics (Figure 8), the study of the physiological effects, shows the percentage of serum hemolytic activity (i.e. the percentage of cell lysis) over time. Cell lysis was dramatically reduced in the majority of the patients to below 20% of normal serum complement activity while under eculizumab treatment. Two patients exhibited a breakthrough in complement activity, but complement blockade was permanently restored by reducing the dosing interval to 12 days (See, Figure 4).

Improvement of quality of life issues was also evaluated using the European Organization for Research and Treatment of Cancer Core (http://www.eortc.be) questionnaires ("EORTC QLC-C30"). Each of the participating patients completed the QLC-30 questionnaire before and during the eculizumab therapy. Overall improvements were observed in global health status, physical functioning, role functioning, emotional functioning, cognitive functioning, fatigue, pain, dyspnea and insomnia. (See Figure 9).

Patients in the two year study experienced a reduction in adverse symptoms associated with PNH. For example, as set forth in Figure 10, there was a demonstrated decrease of abdominal pain, dysphagia, and erectile

dysfunction after administration of eculizumab in those patients reporting those symptoms before administration of eculizumab.

Although preferred and other embodiments of the invention have been described herein, further embodiments may be perceived by those skilled in the art without departing from the scope of the invention.

WHAT IS CLAIMED IS:

1. A method of treating a hemolytic disease in a subject comprising administering an anti-C5 antibody to a subject having a hemolytic disease, wherein there is a reduction in hemoglobinuria in the subject.

2. A method as in claim 1 wherein the anti-C5 antibody is selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

3. A method as in claim 1 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

4. A method as in claim 1 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

5. A method as in claim 1 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

6. A method as in claim 1 wherein the subject's platelet count is greater than 40,000 per microliter.

7. A method as in claim 1 wherein the subject's platelet count is greater than 75,000 per microliter.

8. A method as in claim 1 wherein the subject's platelet count is greater than 150,000 per microliter.

9. A method as in claim 1 wherein the subject's reticulocyte count is greater than 80×10^9 per liter.

10. A method as in claim 1 wherein the subject's reticulocyte count is greater than 120×10^9 per liter.

11. A method as in claim 1 wherein the subject's reticulocyte count is greater than 150×10^9 per liter.

12. A method as in claim 1 wherein said subject has a greater than 40% PNH type III granulocyte clone.

13. A method as in claim 1 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

14. A method of treating a hemolytic disease in a subject comprising administering to a subject having a hemolytic disease a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors, wherein there is a reduction in hemoglobinuria in the subject.

15. A method as in claim 14 wherein the compound is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

16. A method as in claim 14 wherein said subject has a greater than 40% PNH type III granulocyte clone.

17. A method as in claim 14 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

18. A method of restoring nitric oxide (NO) homeostasis comprising administering an anti-C5 antibody to a subject having a hemolytic disease.

19. A method as in claim 18 wherein the anti-C5 antibody is selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

20. A method as in claim 18 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

21. A method as in claim 18 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

22. A method as in claim 18 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

23. A method as in claim 18 wherein the subject's platelet count is greater than 40,000 per microliter.

24. A method as in claim 18 wherein the subject's platelet count is greater than 75,000 per microliter.

25. A method as in claim 18 wherein the subject's platelet count is greater than 150,000 per microliter.

26. A method as in claim 18 wherein the subject's reticulocyte count is greater than 80×10^9 per liter.

27. A method as in claim 18 wherein the subject's reticulocyte count is greater than 120×10^9 per liter.

28. A method as in claim 18 wherein the subject's reticulocyte count is greater than 150×10^9 per liter.

29. A method as in claim 18 wherein said subject has a greater than 40% PNH type III granulocyte clone.

30. A method as in claim 18 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

31. A method of restoring nitric oxide (NO) homeostasis comprising administering to a subject having a hemolytic disease a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

32. A method as in claim 31 wherein the compound is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

33. A method as in claim 31 wherein said subject has a greater than 40% PNH type III granulocyte clone.

34. A method as in claim 31 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

35. A method of reducing hemolysis in a subject having a hemolytic disease comprising administering an anti-C5 antibody, wherein hemolysis is reduced as evidenced by a greater than 50% reduction in lactate dehydrogenase (LDH) levels in the subject's bloodstream.

36. A method as in claim 35 wherein hemolysis is reduced as evidenced by a greater than 65% reduction in lactate dehydrogenase (LDH) levels in the subject's bloodstream.

37. A method as in claim 35 wherein hemolysis is reduced as evidenced by a greater than 80% reduction in lactate dehydrogenase (LDH) levels in the subject's bloodstream.

38. A method as in claim 35 wherein the anti-C5 antibody is selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

39. A method as in claim 35 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

40. A method as in claim 35 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

41. A method as in claim 35 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

42. A method as in claim 35 wherein the subject's platelet count is greater than 40,000 per microliter.

43. A method as in claim 35 wherein the subject's platelet count is greater than 75,000 per microliter.

44. A method as in claim 35 wherein the subject's platelet count is greater than 150,000 per microliter.

45. A method as in claim 35 wherein the subject's reticulocyte count is greater than 80×10^9 per liter.

46. A method as in claim 35 wherein the subject's reticulocyte count is greater than 120×10^9 per liter.

47. A method as in claim 35 wherein the subject's reticulocyte count is greater than 150×10^9 per liter.

48. A method as in claim 35 wherein said subject has a greater than 40% PNH type III granulocyte clone.

49. A method as in claim 35 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

50. A method of reducing hemolysis in a subject having a hemolytic disease comprising administering to the subject a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors, wherein hemolysis is reduced as evidenced by a greater than 50% reduction in lactate dehydrogenase (LDH) levels in the subject's bloodstream.

51. A method as in claim 50 wherein the compound is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

52. A method as in claim 50 wherein said subject has a greater than 40% PNH type III granulocyte clone.

53. A method as in claim 50 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

54. A method of reducing hemoglobinuria in a subject comprising administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement

components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

55. A method as in claim 54 wherein the step of administering comprises administering an anti-C5 antibody.

56. A method as in claim 54 wherein the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

57. A method as in claim 54 wherein the step of administering comprises administering a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

58. A method as in claim 54 wherein the step of administering comprises administering a compound selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

59. A method as in claim 54 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

60. A method as in claim 54 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

61. A method as in claim 54 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

62. A method as in claim 54 wherein the subject's platelet count is greater than 40,000 per microliter.

63. A method as in claim 54 wherein the subject's platelet count is greater than 75,000 per microliter.

64. A method as in claim 54 wherein the subject's platelet count is greater than 150,000 per microliter.

65. A method as in claim 54 wherein the subject's reticulocyte count is greater than 80 x 10^9 per liter.

66. A method as in claim 54 wherein the subject's reticulocyte count is greater than 120 x 10^9 per liter.

67. A method as in claim 54 wherein the subject's reticulocyte count is greater than 150 x 10^9 per liter.

68. A method as in claim 54 wherein said subject has a greater than 40% PNH type III granulocyte clone.

69. A method as in claim 54 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

70. A method of reducing dysphagia in a subject comprising administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components and compounds which block the generation of one or more complement components.

71. A method as in claim 70 wherein the step of administering comprises administering an anti-C5 antibody.

72. A method as in claim 70 wherein the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

73. A method as in claim 70 wherein the step of administering comprises administering a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

74. A method as in claim 70 wherein the step of administering comprises administering a compound selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

75. A method as in claim 70 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

76. A method as in claim 70 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

77. A method as in claim 70 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

78. A method as in claim 70 wherein the subject's platelet count is greater than 40,000 per microliter.

79. A method as in claim 70 wherein the subject's platelet count is greater than 75,000 per microliter.

80. A method as in claim 70 wherein the subject's platelet count is greater than 150,000 per microliter.

81. A method as in claim 70 wherein the subject's reticulocyte count is greater than 80 x 10^9 per liter.

82. A method as in claim 70 wherein the subject's reticulocyte count is greater than 120 x 10^9 per liter.

83. A method as in claim 70 wherein the subject's reticulocyte count is greater than 150 x 10^9 per liter.

84. A method as in claim 70 wherein said subject has a greater than 40% PNH type III granulocyte clone.

85. A method as in claim 70 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

86. A method of reducing erectile dysfunction in a subject comprising administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components and compounds which block the generation of one or more complement components.

87. A method as in claim 86 wherein the step of administering comprises administering an anti-C5 antibody.

88. A method as in claim 86 wherein the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

89. A method as in claim 86 wherein the step of administering comprises administering a compound selected from the group consisting of

antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

90. A method as in claim 86 wherein the step of administering comprises administering a compound selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

91. A method as in claim 86 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

92. A method as in claim 86 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

93. A method as in claim 86 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

94. A method as in claim 86 wherein the subject's platelet count is greater than 40,000 per microliter.

95. A method as in claim 86 wherein the subject's platelet count is greater than 75,000 per microliter.

96. A method as in claim 86 wherein the subject's platelet count is greater than 150,000 per microliter.

97. A method as in claim 86 wherein the subject's reticulocyte count is greater than 80×10^9 per liter.

98. A method as in claim 86 wherein the subject's reticulocyte count is greater than 120×10^9 per liter.

99. A method as in claim 86 wherein the subject's reticulocyte count is greater than 150 x 10^9 per liter.

100. A method as in claim 86 wherein said subject has a greater than 40% PNH type III granulocyte clone.

101. A method as in claim 86 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

102. A method of reducing thrombosis in a subject comprising administering a compound to a subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

103. A method as in claim 102 wherein the step of administering comprises administering an anti-C5 antibody.

104. A method as in claim 102 wherein the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

105. A method as in claim 102 wherein the step of administering comprises administering an anti-C5a receptor antibody.

106. A method as in claim 102 wherein the step of administering comprises administering a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double

stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

107. A method as in claim 102 wherein the step of administering comprises administering a compound selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

108. A method as in claim 102 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

109. A method as in claim 102 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

110. A method as in claim 102 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

111. A method as in claim 102 wherein the subject's platelet count is greater than 40,000 per microliter.

112. A method as in claim 102 wherein the subject's platelet count is greater than 75,000 per microliter.

113. A method as in claim 102 wherein the subject's platelet count is greater than 150,000 per microliter.

114. A method as in claim 102 wherein the subject's reticulocyte count is greater than 80×10^9 per liter.

115. A method as in claim 102 wherein the subject's reticulocyte count is greater than 120×10^9 per liter.

116. A method as in claim 102 wherein the subject's reticulocyte count is greater than 150×10^9 per liter.

117. A method as in claim 102 wherein said subject has a greater than 40% PNH type III granulocyte clone.

118. A method as in claim 102 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

119. A method of increasing proportion of type III red blood cells of a subject's total red blood cell content comprising administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

120. A method as in claim 119 wherein the step of administering comprises administering an anti-C5 antibody.

121. A method as in claim 119 wherein the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

122. A method as in claim 119 wherein the step of administering comprises administering a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

123. A method as in claim 119 wherein the step of administering comprises administering a compound selected from the group consisting of

CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

124. A method as in claim 119 wherein the amount of PNH type III red blood cells is greater than about 10% of the subject's total red blood cell count.

125. A method as in claim 119 wherein the amount of PNH type III red blood cells is greater than about 25% of the subject's total red blood cell count.

126. A method as in claim 119 wherein the amount of PNH type III red blood cells is greater than about 50% of the subject's total red blood cell count.

127. A method as in claim 119 wherein the amount of PNH type III red blood cells is greater than about 75% of the subject's total red blood cell count.

128. A method as in claim 119 wherein the subject's platelet count is greater than 40,000 per microliter.

129. A method as in claim 119 wherein the subject's platelet count is greater than 75,000 per microliter.

130. A method as in claim 119 wherein the subject's platelet count is greater than 150,000 per microliter.

131. A method as in claim 119 wherein the subject's reticulocyte count is greater than 80×10^9 per liter.

132. A method as in claim 119 wherein the subject's reticulocyte count is greater than 120×10^9 per liter.

133. A method as in claim 119 wherein the subject's reticulocyte count is greater than 150 x 10^9 per liter.

134. A method as in claim 119 wherein said subject has a greater than 40% PNH type III granulocyte clone.

135. A method as in claim 119 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

136. A method of reducing hemolysis in a subject comprising administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

137. A method as in claim 136 wherein the step of administering comprises administering an anti-C5 antibody.

138. A method as in claim 136 wherein the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

139. A method as in claim 136 wherein the step of administering comprises administering a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

140. A method as in claim 136 wherein the step of administering comprises administering a compound selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

141. A method as in claim 136 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

142. A method as in claim 136 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

143. A method as in claim 136 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

144. A method as in claim 136 wherein the subject's platelet count is greater than 40,000 per microliter.

145. A method as in claim 136 wherein the subject's platelet count is greater than 75,000 per microliter.

146. A method as in claim 136 wherein the subject's platelet count is greater than 150,000 per microliter.

147. A method as in claim 136 wherein the subject's reticulocyte count is greater than 80 x 10^9 per liter.

148. A method as in claim 136 wherein the subject's reticulocyte count is greater than 120 x 10^9 per liter.

149. A method as in claim 136 wherein the subject's reticulocyte count is greater than 150 x 10^9 per liter.

150. A method as in claim 136 wherein said subject has a greater than 40% PNH type III granulocyte clone.

151. A method as in claim 136 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

152. A method of treating a nitric oxide (NO) deficiency in a subject afflicted with a hemolytic disease comprising administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

153. A method as in claim 152 wherein the step of administering comprises administering an anti-C5 antibody.

154. A method as in claim 152 wherein the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

155. A method as in claim 152 wherein the step of administering comprises administering a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

156. A method as in claim 152 wherein the step of administering comprises administering a compound selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

157. A method as in claim 152 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

158. A method as in claim 152 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

159. A method as in claim 152 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

160. A method as in claim 152 wherein the subject's platelet count is greater than 40,000 per microliter.

161. A method as in claim 152 wherein the subject's platelet count is greater than 75,000 per microliter.

162. A method as in claim 152 wherein the subject's platelet count is greater than 150,000 per microliter.

163. A method as in claim 152 wherein the subject's reticulocyte count is greater than 80×10^9 per liter.

164. A method as in claim 152 wherein the subject's reticulocyte count is greater than 120×10^9 per liter.

165. A method as in claim 152 wherein the subject's reticulocyte count is greater than 150×10^9 per liter.

166. A method as in claim 152 wherein said subject has a greater than 40% PNH type III granulocyte clone.

167. A method as in claim 152 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

168. A method of rendering a subject afflicted with a hemolytic disease transfusion independent comprising administering a compound to the

subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

169. A method as in claim 168 wherein the step of administering comprises administering an anti-C5 antibody.

170. A method as in claim 168 wherein the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

171. A method as in claim 168 wherein the step of administering comprises administering a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

172. A method as in claim 168 wherein the step of administering comprises administering a compound selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

173. A method as in claim 168 further comprising the step of administering one or more compounds that increase hematopoiesis in combination with said compound.

174. A method as in claim 173 wherein the one or more compounds that increase hematopoiesis is selected from the group consisting of steroids,

immunosuppressants, anti-coagulants, folic acid, iron, erythropoietin (EPO), antithymocyte globulin (ATG) and antilymphocyte globulin (ALG).

175. A method as in claim 173 wherein EPO is administered in combination with an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

176. A method as in claim 168 wherein the subject is transfusion independent for over six months from the first administration of said compound.

177. A method as in claim 168 wherein the subject is transfusion independent for over twelve months from the first administration of said compound.

178. A method as in claim 168 wherein the subject is transfusion independent for over two years from the first administration of said compound.

179. A method as in claim 168 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

180. A method as in claim 168 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

181. A method as in claim 168 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

182. A method as in claim 168 wherein the subject's platelet count is greater than 40,000 per microliter.

183. A method as in claim 168 wherein the subject's platelet count is greater than 75,000 per microliter.

184. A method as in claim 168 wherein the subject's platelet count is greater than 150,000 per microliter.

185. A method as in claim 168 wherein the subject's reticulocyte count is greater than 80 x 10^9 per liter.

186. A method as in claim 168 wherein the subject's reticulocyte count is greater than 120 x 10^9 per liter.

187. A method as in claim 168 wherein the subject's reticulocyte count is greater than 150 x 10^9 per liter.

188. A method as in claim 168 wherein said subject has a greater than 40% PNH type III granulocyte clone.

189. A method as in claim 168 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

190. A method of treating a subject afflicted with a hemolytic disease comprising administering: 1) one or more compounds selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components; in combination with 2) one or more compounds that increase hematopoiesis.

191. A method as in claim 190 wherein the one or more compounds that increase hematopoiesis are selected from the group consisting of steroids, immunosuppressants, anti-coagulants, folic acid, iron, erythropoietin (EPO), antithymocyte globulin (ATG) and antilymphocyte globulin (ALG).

192. A method as in claim 190 wherein EPO is administered in combination with an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

193. A method as in claim 190 wherein EPO is administered in combination with a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

194. A method as in claim 190 wherein EPO is administered in combination with a compound selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

195. A method as in claim 190 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

196. A method as in claim 190 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

197. A method as in claim 190 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

198. A method as in claim 190 wherein the subject's platelet count is greater than 40,000 per microliter.

199. A method as in claim 190 wherein the subject's platelet count is greater than 75,000 per microliter.

200. A method as in claim 190 wherein the subject's platelet count is greater than 150,000 per microliter.

201. A method as in claim 190 wherein the subject's reticulocyte count is greater than 80×10^9 per liter.

202. A method as in claim 190 wherein the subject's reticulocyte count is greater than 120 x 10^9 per liter.

203. A method as in claim 190 wherein the subject's reticulocyte count is greater than 150 x 10^9 per liter.

204. A method as in claim 190 wherein said subject has a greater than 40% PNH type III granulocyte clone.

205. A method as in claim 190 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

206. A method of reducing hemolysis in a subject having a hemolytic disease comprising administering an anti-C5 antibody, wherein hemolysis is reduced as evidenced by a reduction in lactate dehydrogenase (LDH) levels in the subject's bloodstream to within 20% of the upper limit of normal.

207. A method as in claim 206 wherein the anti-C5 antibody is selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

208. A method as in claim 206 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

209. A method as in claim 206 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

210. A method as in claim 206 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

211. A method as in claim 206 wherein the subject's platelet count is greater than 40,000 per microliter.

212. A method as in claim 206 wherein the subject's platelet count is greater than 75,000 per microliter.

213. A method as in claim 206 wherein the subject's platelet count is greater than 150,000 per microliter.

214. A method as in claim 206 wherein the subject's reticulocyte count is greater than 80 x 10^9 per liter.

215. A method as in claim 206 wherein the subject's reticulocyte count is greater than 120 x 10^9 per liter.

216. A method as in claim 206 wherein the subject's reticulocyte count is greater than 150 x 10^9 per liter.

217. A method as in claim 206 wherein said subject has a greater than 40% PNH type III granulocyte clone.

218. A method as in claim 206 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

219. A method of reducing hemolysis in a subject having a hemolytic disease comprising administering to the subject a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors, wherein hemolysis is reduced as evidenced by a reduction in lactate dehydrogenase (LDH) levels in the subject's bloodstream to within 20% of the upper limit of normal.

220. A method as in claim 219 wherein the compound is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

221. A method as in claim 219 wherein said subject has a greater than 40% PNH type III granulocyte clone.

222. A method as in claim 219 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

223. A method of reducing hemolysis in a subject having a hemolytic disease comprising administering an anti-C5 antibody, wherein serum complement hemolytic activity is reduced at least 80% as evidenced by a serum hemolytic assay.

224. A method as in claim 223 wherein the anti-C5 antibody is selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

225. A method as in claim 223 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

226. A method as in claim 223 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

227. A method as in claim 223 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

228. A method as in claim 223 wherein the subject's platelet count is greater than 40,000 per microliter.

229. A method as in claim 223 wherein the subject's platelet count is greater than 75,000 per microliter.

230. A method as in claim 223 wherein the subject's platelet count is greater than 150,000 per microliter.

231. A method as in claim 223 wherein the subject's reticulocyte count is greater than 80 x 10^9 per liter.

232. A method as in claim 223 wherein the subject's reticulocyte count is greater than 120 x 10^9 per liter.

233. A method as in claim 223 wherein the subject's reticulocyte count is greater than 150 x 10^9 per liter.

234. A method as in claim 223 wherein said subject has a greater than 40% PNH type III granulocyte clone.

235. A method as in claim 223 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

236. A method of reducing hemolysis in a subject having a hemolytic disease comprising administering to the subject a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors, wherein serum complement hemolytic activity is reduced at least 80% as evidenced by a serum hemolytic assay.

237. A method as in claim 236 wherein the compound is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

238. A method as in claim 236 wherein said subject has a greater than 40% PNH type III granulocyte clone.

239. A method as in claim 236 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

240. A method of reducing abdominal pain in a subject comprising administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

241. A method as in claim 240 wherein the step of administering comprises administering an anti-C5 antibody.

242. A method as in claim 240 wherein the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

243. A method as in claim 240 wherein the step of administering comprises administering a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

244. A method as in claim 240 wherein the step of administering comprises administering a compound selected from the group consisting of

CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

245. A method as in claim 240 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

246. A method as in claim 240 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

247. A method as in claim 240 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

248. A method as in claim 240 wherein the subject's platelet count is greater than 40,000 per microliter.

249. A method as in claim 240 wherein the subject's platelet count is greater than 75,000 per microliter.

250. A method as in claim 240 wherein the subject's platelet count is greater than 150,000 per microliter.

251. A method as in claim 240 wherein the subject's reticulocyte count is greater than 80×10^9 per liter.

252. A method as in claim 240 wherein the subject's reticulocyte count is greater than 120×10^9 per liter.

253. A method as in claim 240 wherein the subject's reticulocyte count is greater than 150×10^9 per liter.

254. A method as in claim 240 wherein said subject has a greater than 40% PNH type III granulocyte clone.

255. A method as in claim 240 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

256. A method of decreasing the frequency of transfusions required by a subject afflicted with a hemolytic disease comprising administering a compound to the subject, the compound being selected from the group consisting of compounds which bind to one or more complement components, compounds which block the generation of one or more complement components and compounds which block the activity of one or more complement components.

257. The method of claim 256 wherein the frequency of transfusions is decreased by about 50%.

258. The method of claim 256 wherein the frequency of transfusions is decreased by about 70%.

259. The method of claim 256 wherein the frequency of transfusions is decreased by about 90%.

260. A method as in claim 256 wherein the step of administering comprises administering an anti-C5 antibody.

261. A method as in claim 256 wherein the step of administering comprises administering an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

262. A method as in claim 256 wherein the step of administering comprises administering a compound selected from the group consisting of antibodies, soluble complement inhibitory compounds, proteins, protein fragments, peptides, small molecules, RNA aptamers, L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors.

263. A method as in claim 256 wherein the step of administering comprises administering a compound selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

264. A method as in claim 256 further comprising the step of administering one or more compounds that increase hematopoiesis in combination with said compound.

265. A method as in claim 264 wherein the one or more compounds that increase hematopoiesis is selected from the group consisting of steroids, immunosuppressants, anti-coagulants, folic acid, iron, erythropoietin (EPO), antithymocyte globulin (ATG) and antilymphocyte globulin (ALG).

266. A method as in claim 264 wherein EPO is administered in combination with an anti-C5 antibody selected from the group consisting of h5G1.1-mAb, h5G1.1-scFv and functional fragments of h5G1.1.

267. A method as in claim 256 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 10%.

268. A method as in claim 256 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 25%.

269. A method as in claim 256 wherein the proportion of type III red blood cells of the subject's total red blood cell content is greater than 50%.

270. A method as in claim 256 wherein the subject's platelet count is greater than 40,000 per microliter.

271. A method as in claim 256 wherein the subject's platelet count is greater than 75,000 per microliter.

272. A method as in claim 256 wherein the subject's platelet count is greater than 150,000 per microliter.

273. A method as in claim 256 wherein the subject's reticulocyte count is greater than 80 x 10^9 per liter.

274. A method as in claim 256 wherein the subject's reticulocyte count is greater than 120 x 10^9 per liter.

275. A method as in claim 256 wherein the subject's reticulocyte count is greater than 150 x 10^9 per liter.

276. A method as in claim 256 wherein said subject has a greater than 40% PNH type III granulocyte clone.

277. A method as in claim 256 wherein said subject has an LDH level greater than or equal to 1.5 times the upper limit of a normal LDH level in a person.

Figure 1A.
Biochemical Parameters of Hemolysis During Eculizumab Treatment

Biochemical Marker	Normal range	Time of Analysis			p-value[a]
		Pre-study[b]	12 weeks	64 weeks	
LDH (IU/L)	150 - 480	3110.7 +/- 598.4	594.0 +/- 31.7	622.4 +/-41.1	0.002
AST (IU/L)	10 - 40	76.2 +/- 16.0	26.2 +/- 2.3	30.1 +/- 3.2	0.02
Haptoglobin (g/L)	0.5 - 2	<0.06	<0.06	0.14 +/- 0.07[c]	N.S.[d]
Hemoglobin (g/dL)	11.5 - 18	10.0 +/- 0.4	10.3 +/- 0.4	10.4 +/- 0.4	N.S.
Bilirubin	3 - 15	25.9 +/- 4.3	28.2 +/- 4.4	28.7 +/- 4.0	N.S.
Reticulocytes (x10^3/mm^3)	20 - 80	161.4 +/- 25.9	191.2 +/- 23.6	189.6 +/- 21.8	N.S.

[a]from comparisons of mean change from pre-study to 64 weeks
[b]values represent means during 52 week period prior to treatment except for AST which represents the baseline mean
[c]10 of 11 patients were below the detectable limit of haptoglobin (<0.06 g/L); 1 patient had a value of 0.69 g/L.
[d]not significant

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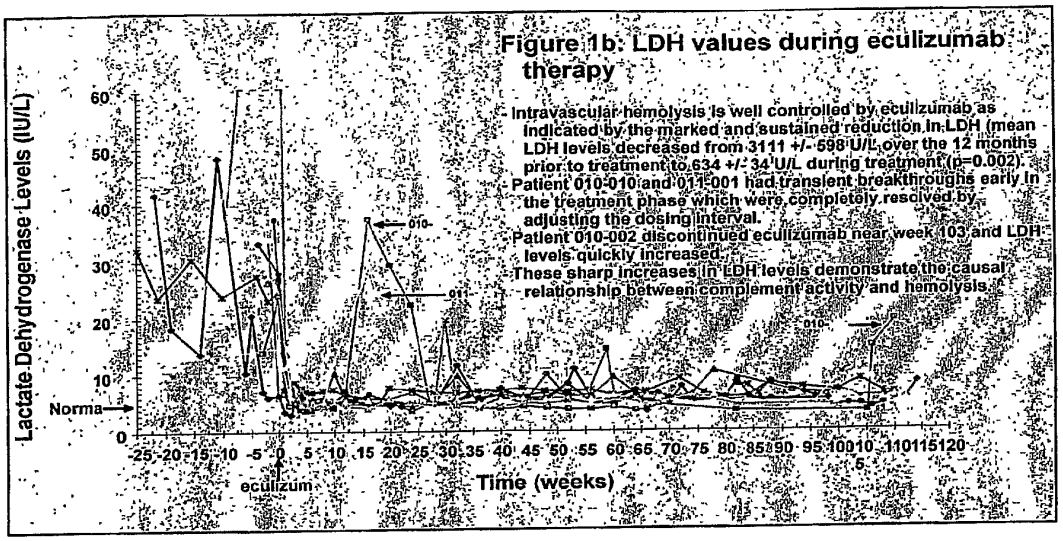
Figure 1b



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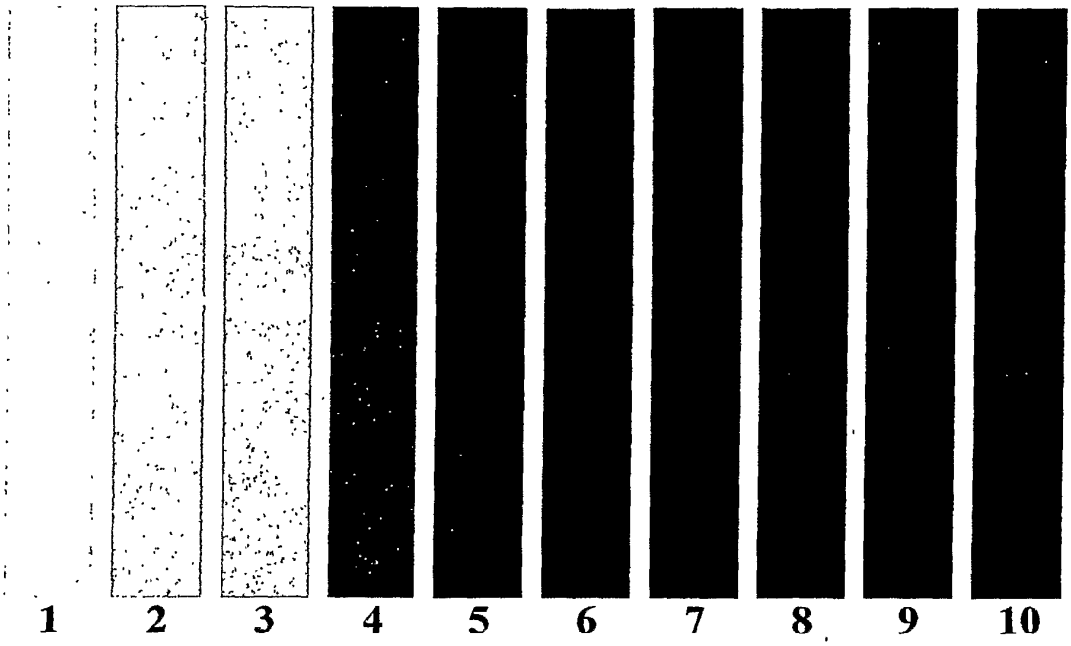

Figure 2

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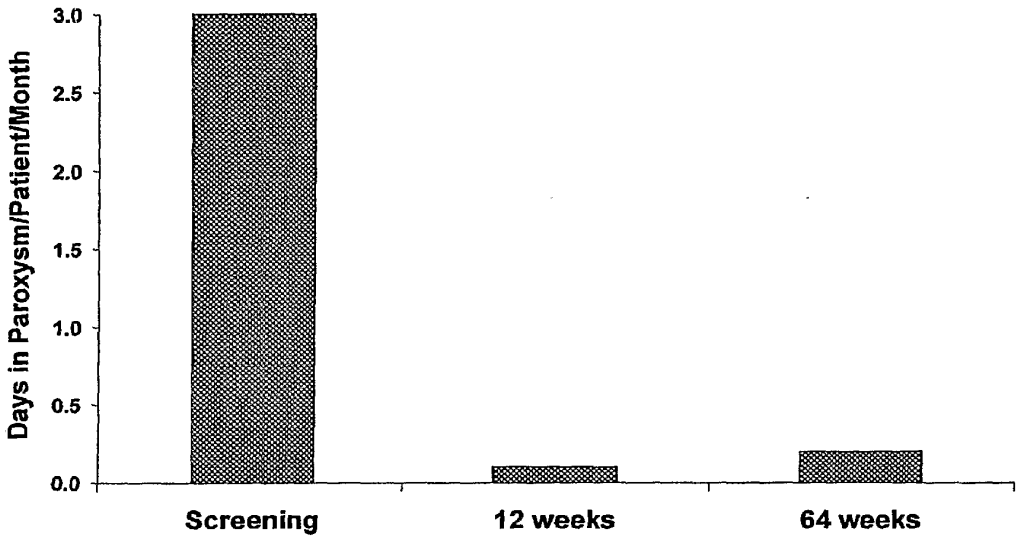
Figure 3.
Effect of Eculizumab on Paroxysm Rate (n=8)



Bars represent the paroxysm rates (number of paroxysms per patient per month) during the screening period (pre-eculizumab treatment), and during the first 12 weeks and the entire 64 weeks of eculizumab treatment. Three patients were not included in the analysis as either pretreatment urine scores were inadvertently not collected (2 patients) or an iron chelating agent that resulted in artificially colored urine was administered during the extension study (1 patient).

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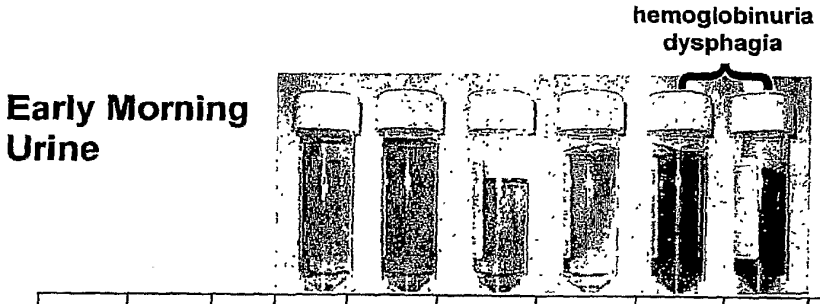
Figure 4.
Analysis of Complement Breakthrough



Visit	4							5			9
Day	0*	1-8	9	10	11	12	13	0*	1-2		0*
Urine	10	2-3	3	3	3	3	9	10	3		2
LDH	2624	–	784	–	–	697	1687	2917	–		495
AST	119	–	38	–	–	31	87	–	–		–
PK	28	–	51	–	–	35	31	23	–		51
PD	72	–	1	–	–	2	56	56	–		2

*Dose of eculizumab

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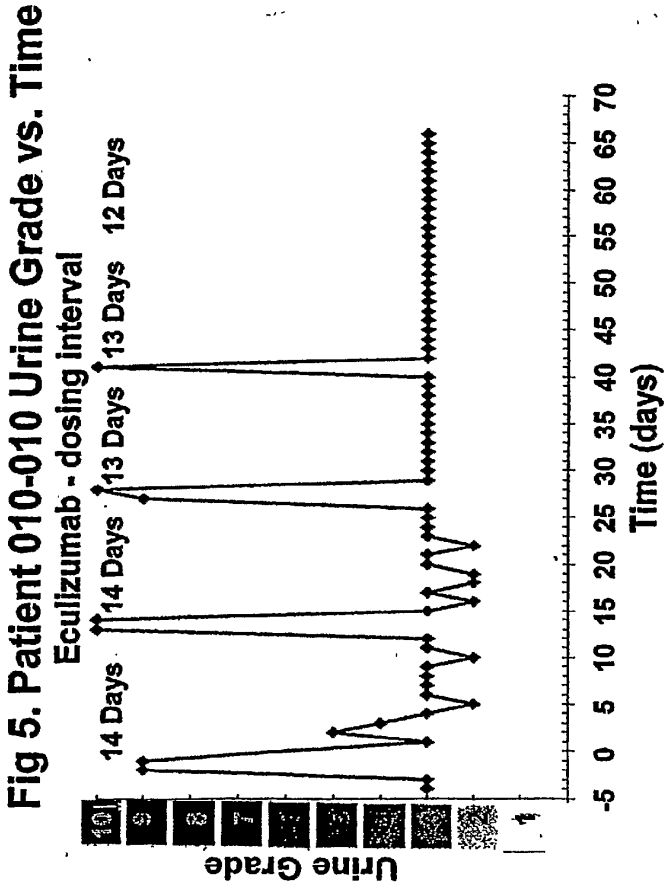

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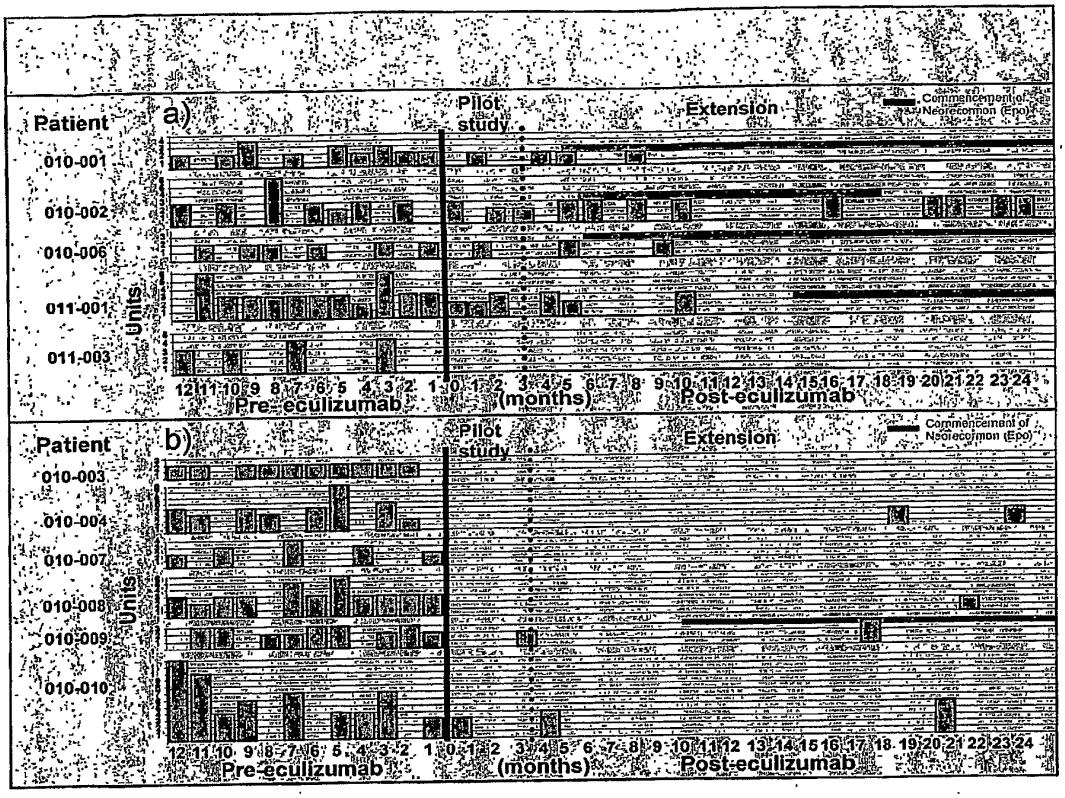
Figure 6.
Units Transfused Pre- and Post-Eculizumab in (a) Cytopenic Patients and (b) Non-Cytopenic Patients



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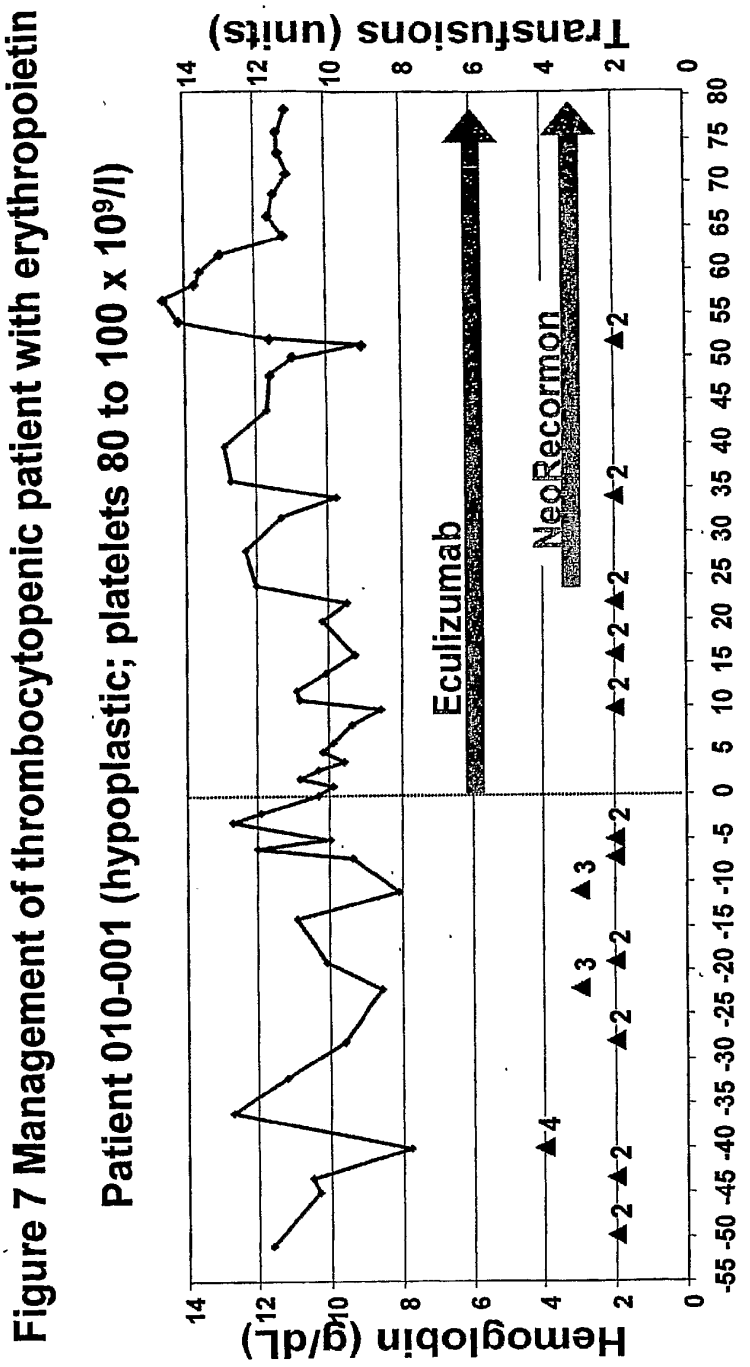

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Figure 8.
Eculizumab Pharmacodynamics Pre- and Post-dose

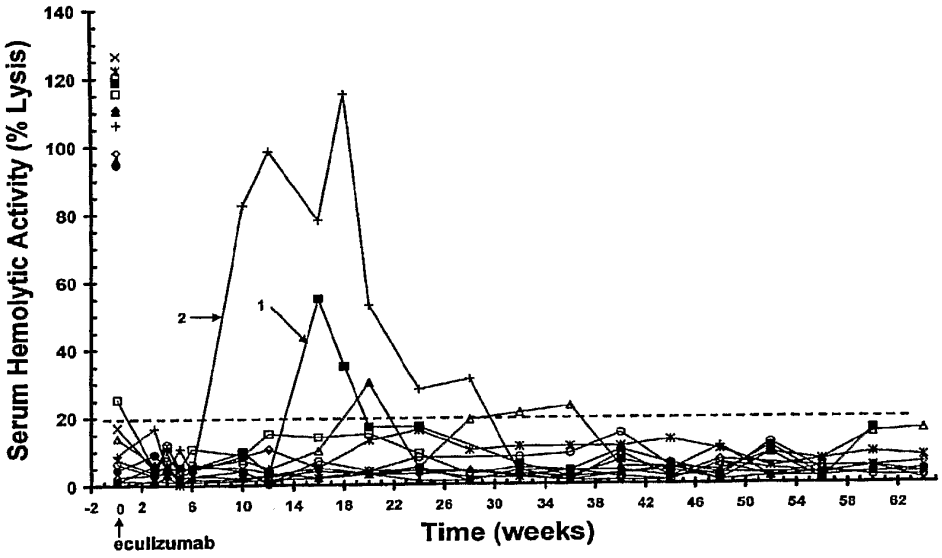

Figure 8 shows serum hemolytic activity (PD) during the 64 week treatment period as determined by the ability of serum to lyse antibody-presensitized chicken erythrocytes. The percentage of hemolytic activity at which complement is considered to be effectively inhibited (≤ 20 %) is indicated by dashed line. Two patients that demonstrated trough serum hemolytic activity values less than 20% are identified (patients 1 and 2).

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Figure 9.
Eculizumab Pharmacodynamics Pre- and Post-dose

Domain (a)	Mean Base-Line Score (b)	64 Week Change from Base-line (c)	p-value (d)
Global Health Status	56.1	13.8	0.009
Physical Functioning	70.9	14.3	<0.001
Emotional Functioning	70.5	12.5	<0.001
Role Functioning	66.7	14.5	0.003
Cognitive Functioning	77.3	10.3	0.001
Fatigue	47.5	-17.8	<0.001
Dyspnea	39.4	-16.6	0.002
Insomnia	30.3	-8.2	0.031
Pain	21.2	-8.2	0.023
Constipation	3.0	4.1	<0.001

a) the quality of life was assessed using the European Organization for Research and Treatment of Cancer QLQ-C30 instrument
b) numbers represent mean values of linearly transformed scores
c) values represent least-square means; positive change indicates improvement for Global Health Status and Functional Scales while a negative change indicates improvement for Symptom Scales
d) values are from a mixed analysis-of-covariance model with visit as a fixed effect, patient as a random effect, and baseline as a covariate

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Figure 10.
Symptoms Pre- and 2 Years Post-Eculizumab

Patient	Pre-Eculizumab			Post-Eculizumab		
	Abdominal Pain	Dysphagia	Erectile Dysfunction	Abdominal Pain	Dysphagia	Erectile Dysfunction
010-001	Every 4 – 8 weeks	Every 4 – 8 weeks	Every 4 – 8 weeks	None	None	None
010-002	None	None	--	None	None	--
010-003	Every week	Every week	Every week	None	None	None
010-004	Every 4 – 6 weeks	None	--	None	None	--
010-006	None	None	None	None	None	None
010-007	None	At least every 4 weeks	--	None	None	--
010-008	None	Every 10 weeks	Every 10 weeks	None	None	Intermittent
010-009	None	None	None	None	None	None
010-010	Every 4 weeks	Every 4 weeks	None	None	2 episodes in 2 years*	None
011-001	None	None	--	None	None	--
011-003	None	None	--	None	None	--

*This patient experienced a transient breakthrough in complement blockade with a return of hemolysis and symptoms. Increasing the dosing frequency to 12 days re-established complete complement blockade and prevented further symptoms.

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Filing Date	2016-09-09
First Named Inventor	Leonard BELL
Art Unit	1644
Examiner Name	J. L. Rogers
Attorney Docket Number	AXJ-114BUSCN3

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	2	5135916		1992-08-04	Sims et al.	
	3	5660825		1997-08-26	Sims et al.	
	4	6355245		2002-03-12	Evans et al.	
	5	7361339		2008-04-22	Bell	
	6	7482435		2009-01-27	Bowdish et al.	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

	1	20030049260	A1	2003-03-13	Bell	
	2	20030049683	A1	2003-03-13	Bowdish et al.	
	3	20040219147	A1	2004-11-04	Bell	
	4	20040038308		2004-02-26	Rother et al.	
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	7	20050191298	A1	2005-09-01	Bell et al.	
	8	20050221382	A1	2005-10-06	Rother	
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	12	20060057679	A1	2006-03-16	O'Keefe et al.	
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	20	20160244516	A1	2016-08-25	BELL et al.	

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	1	2007005612	WO	A2	2007-01-11	Medimmune, Inc		
	2	2007002571	WO	A2	2007-01-04	Alexion Pharmaceuticals, Inc		
	3	2005110481	WO	A2	2005-11-24	Alexion Pharmaceuticals, Inc		
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	5	2004/029207	WO	A2	2004-04-08	Xencor Inc		
	6	0411306	EP	A1	1991-02-06	GOETZE OTTO		X

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	1	Adis International Limited, "Eculizumab: 5G1.1, h5G1.1, Long-Acting Anti-C5 Monoclonal Antibody 5G1-1, Long-Acting Anti-C5 Monoclonal Antibody 5G1.1," Drugs in Research and Development, vol. 8 (1): 61-68 (2007).	
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EXAMINER SIGNATURE

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

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CERTIFICATION STATEMENT

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

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

OR

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

See attached certification statement.

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

☒ A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Jill Gorny Sloper/	Date (YYYY-MM-DD)	2016-10-21
Name/Print	Jill Gorny Sloper, Esq.	Registration Number	60,760

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

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3	2558

133485 7590 10/27/2016

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
10/27/2016	ELECTRONIC

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

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

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

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 15/260,888	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	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* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

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

Status

1)☒ Responsive to communication(s) filed on *9/9/2016*.

☐ 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) *1-10* 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) *1-10* 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/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *10/21/2016*.

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

4) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-10 pending and under consideration in the instant examination.

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

Double Patenting

3. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., In re Berg, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); In re Goodman, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); In re Van Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); In re Thorington, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

4. Claims 1-10 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claim 1 of copending Application No. 15/148,839, in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9).

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because the '839 application claims a pharmaceutical composition for use in treating a patient afflicted with PNH. The specification of the '516 at ¶158 defines eculizumab as having a heavy chain SEQ ID NO: 2 and light chain SEQ ID NO: 4 which is 100% identical in amino acid sequence to the currently claimed SEQ IDs NO: 2 and 4. (see alignment below).

SEQ ID NO: 2 alignment

```
Qy          1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY 60

Qy         61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV 120

Qy        121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ 180

Qy        181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS 240

Qy        241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST 300

Qy        301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT 360

Qy        361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE 420

Qy        421 GNVFSCSVMHEALHNHYTQKSLSLSLGK 448
              ||||||||||||||||||||||||||||
Db        421 GNVFSCSVMHEALHNHYTQKSLSLSLGK 448
```

SEQ ID NO: 4 alignment

```
Qy          1 MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQ 60
```

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                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQ 60

Qy         61 KPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNVLNTPLTF 120
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 KPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNVLNTPLTF 120

Qy        121 GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN 180
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN 180

Qy        181 SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 236
                 |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 236
```

While the ‘839 does not specifically recite that the patient is anemic or that the antibody is administered to the patient at a dosage level of between 5-50 mg per kg per patient per treatment, in a single unit dosage form of the other various dosage schedules currently recited (e.g, 600 m/900/900 mg), Hillman teaches administration of the anti-C5 antibody eculizumab to an anemic patient (p. 555 under “demographic characteristics” at a dosing schedule of 600 mg for four weeks, followed one week later by a 900 mg dose and then by a dose of 900 mg every other week through week 12 (p. 554, under “treatment schedule”). In addition, [I]t is not inventive to discover the optimum or workable ranges by routine experimentation.” In re Aller, 220 F.2d 454, 456 (CCPA 1955); see also In re Peterson, 315 F.3d 1325 (Fed. Cir. 2003). “Only if the ‘results of optimizing a variable’ are ‘unexpectedly good’ can a patent be obtained for the claimed critical range.” In re Geisler, 116 F.3d 1465, 1469 (Fed. Cir. 1997) (quoting In re Antonie, 559 F.2d 618, 620 (CCPA 1977)). “[Discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art.” In re Boesch, 617 F.2d 272, 276 (CCPA 1980). Given the teachings of Hillman one of skill in the art would regard administration of the anti-C5 antibody recited in '839 as an optimization variable.

5. Claims 1-10 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 91, 98, 100-106, 110-113, and 115 of copending Application No. 15/148,839.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because the ‘973 application claims a method for treating a patient afflicted with PNH by administration of an anti-C5 antibody in single unit dosage form and at a dosing schedule of 600 for the first 4 weeks, 900 mg for the fifth and 900 mg every 14 days thereafter. The specification of the ‘839 (¶s 20, 158) defines an anti-C5 antibody as having a heavy chain SEQ ID NO: 2 and light chain SEQ ID NO: 4 which is 100% identical in amino acid sequence to the currently claimed SEQ IDs NO: 2 and 4. (see alignment below).

SEQ ID NO: 2 alignment

```
Qy          1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY 60

Qy         61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV 120

Qy        121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ 180

Qy        181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS 240

Qy        241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST 300

Qy        301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT 360

Qy        361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE 420

Qy        421 GNVFSCSVMHEALHNHYTQKSLSLSLGK 448
              ||||||||||||||||||||||||||||
Db        421 GNVFSCSVMHEALHNHYTQKSLSLSLGK 448
```

SEQ ID NO: 4 alignment

```
Qy          1 MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQ 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQ 60

Qy         61 KPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNVLNTPLTF 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 KPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNVLNTPLTF 120

Qy        121 GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN 180

Qy        181 SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 236
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 236
```

6. The closest prior art was regarded to be Hillmen et al. (N Engl J Med 2004; 350: 552-9). Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of

eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶). Hillmen teaches that patients received infusions of 600 mg eculizumab weekly for four weeks, followed one week later by a 900 mg dose and then by a dose of 900 mg every other week through week 12 (p. 554 under “treatment schedule”). However, Hillmen does not teach the specific anti-C5 antibody recited by the current application. As evidenced by Report from Alexion Pharma K.K. eculizumab has a H and L chain sequence (p. 3 of the Report) which when compared to the H and L chains of the currently recited anti-C5 antibody are not the same (see sequence alignment below ExPASy (userSeq1 is the imputed sequence of eculizumab and userSeq2 is the amino acid sequence for the H chain of the currently recited claims).

```
98.9% identity in 448 residues overlap; Score: 2364.0; Gap frequency: 0.0%

UserSeq1       1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY
UserSeq2       1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY
                 ************************************************************

UserSeq1      61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV
UserSeq2      61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV
                 ************************************************************

UserSeq1     121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
UserSeq2     121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
                 ************************************************************

UserSeq1     181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS
UserSeq2     181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS
                 ************************************************************

UserSeq1     241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
UserSeq2     241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHANKTKPREEQFNST
                 ********************************************  ************

UserSeq1     301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
UserSeq2     301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPEPEQVYTLPPSQEEMT
                 ******************************************   *************

UserSeq1     361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE
UserSeq2     361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE
                 ************************************************************

UserSeq1     421 GNVFSCSVMHEALHNHYTQKSLSLSLGK
UserSeq2     421 GNVFSCSVMHEALHNHYTQKSLSLSLGK
                 ****************************
```

One of skill in the art would not have been easily guided to using the currently claimed anti-C5 antibody for treating a patient suffering from PNH given the teachings of Hillmen.

7. No claim is allowed.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

October 20, 2016

/JAMES ROGERS/

/SHARON WEN/
Primary Examiner, Art Unit 1644

Notice of References Cited	Application/Control No. 15/260,888	Applicant(s)/Patent Under Reexamination BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-2016/0244516 A1	08-2016	BELL; Leonard	A61K31/7105	1/1
*	B	US-2012/0237515 A1	09-2012	Bell; Leonard	A61K31/7105	424/135.1
	C	US-				
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

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

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Hillmen et al. "Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria" NEJM, 350(6), 2004, pp. 552-559
	V	Alexion Pharma K.K. "Report on the Deliberation Results" for "Soliris for intravenous infusion 300 mg", Marh 5, 2010, pp. 1-105
	W	
	X	

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

ORIGINAL ARTICLE

Effect of Eculizumab on Hemolysis and Transfusion Requirements in Patients with Paroxysmal Nocturnal Hemoglobinuria

Peter Hillmen, M.B., Ph.D., Claire Hall, M.B., Ch.B., Judith C.W. Marsh, M.B., M.D., Modupe Elebute, M.B., M.D., Michael P. Bombara, B.S., Beth E. Petro, B.S., Matthew J. Cullen, B.Sc., Stephen J. Richards, Ph.D., Scott A. Rollins, Ph.D., Christopher F. Mojcik, M.D., Ph.D., and Russell P. Rother, Ph.D.

ABSTRACT

BACKGROUND

Paroxysmal nocturnal hemoglobinuria (PNH) arises from a somatic mutation of the *PIG-A* gene in a hematopoietic stem cell and the subsequent production of blood cells with a deficiency of surface proteins that protect the cells against attack by the complement system. We tested the clinical efficacy of eculizumab, a humanized antibody that inhibits the activation of terminal complement components, in patients with PNH.

METHODS

Eleven transfusion-dependent patients with PNH received infusions of eculizumab (600 mg) every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12. Clinical and biochemical indicators of hemolysis were measured throughout the trial.

RESULTS

Mean lactate dehydrogenase levels decreased from 3111 IU per liter before treatment to 594 IU per liter during treatment (P=0.002). The mean percentage of PNH type III erythrocytes increased from 36.7 percent of the total erythrocyte population to 59.2 percent (P=0.005). The mean and median transfusion rates decreased from 2.1 and 1.8 units per patient per month to 0.6 and 0.0 units per patient per month, respectively (P=0.003 for the comparison of the median rates). Episodes of hemoglobinuria were reduced by 96 percent (P<0.001), and measurements of the quality of life improved significantly.

CONCLUSIONS

Eculizumab is safe and well tolerated in patients with PNH. This antibody against terminal complement protein C5 reduces intravascular hemolysis, hemoglobinuria, and the need for transfusion, with an associated improvement in the quality of life in patients with PNH.

From the Department of Haematology, Leeds Teaching Hospitals National Health Science Trust, Leeds, United Kingdom (P.H., C.H., M.J.C., S.J.R.); the Department of Haematology, St. George's Hospital Medical School, London (J.C.W.M., M.E.); and Alexion Pharmaceuticals, Cheshire, Conn. (M.P.B., B.E.P., S.A.R., C.F.M., R.P.R.). Address reprint requests to Dr. Hillmen at the Department of Haematology, Leeds General Infirmary, Great George St., Leeds LS1 3EX, United Kingdom, or at peter.hillmen@panp-tr.northy.nhs.uk.

N Engl J Med 2004;350:552-9.

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THE MAJOR CLINICAL SIGNS OF PAROXysmal nocturnal hemoglobinuria (PNH) are intravascular hemolysis, venous thrombosis, and hemoglobinuria.[1] The disease arises from a somatic mutation of the *PIG-A* gene in a pluripotent hematopoietic stem cell. *PIG-A* encodes a protein that is essential for the synthesis of glycosylphosphatidylinositol (GPI), a lipid moiety that is embedded in the plasma membrane, where it serves to anchor a wide variety of proteins to the cell surface. The mutant stem cell subsequently expands to form a hematopoietic clone with a deficiency in proteins that are normally attached to the cell surface by the GPI anchor.[2,3] The mature blood cells derived from the hematopoietic clone can have a complete deficiency (type III) or a partial deficiency (type II) of GPI-linked proteins and almost always coexist with residual cells with a normal expression of these proteins (previously identified as type I).

The clinical features of PNH result from the lack of one or more GPI-linked proteins that protect cells from complement-mediated attack. Two such proteins — CD55 and CD59 — are absent from PNH type III erythrocytes, platelets, and other blood cells.[4-6] CD55 regulates early complement activation by inhibiting C3 convertases,[7] whereas CD59 inhibits the assembly of the membrane-attack complex C5b–C9 by interacting with C8 and C9.[4,5] The lack of CD59 is probably responsible for the increased sensitivity of PNH erythrocytes and platelets to complement.[4,8-13]

Eculizumab is a recombinant humanized monoclonal antibody that was designed to block the activation of terminal complement components.[14,15] It binds specifically to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b, thereby preventing the release of the inflammatory mediator C5a and the formation of the cytolytic-pore C5b–C9. Blockade of the complement cascade at C5 preserves the early components of complement that are essential for the opsonization of microorganisms and clearance of immune complexes.[16] In this trial, we investigated whether eculizumab could reduce the incidence of intravascular hemolysis, hemoglobinuria, and transfusion requirements in patients with PNH.

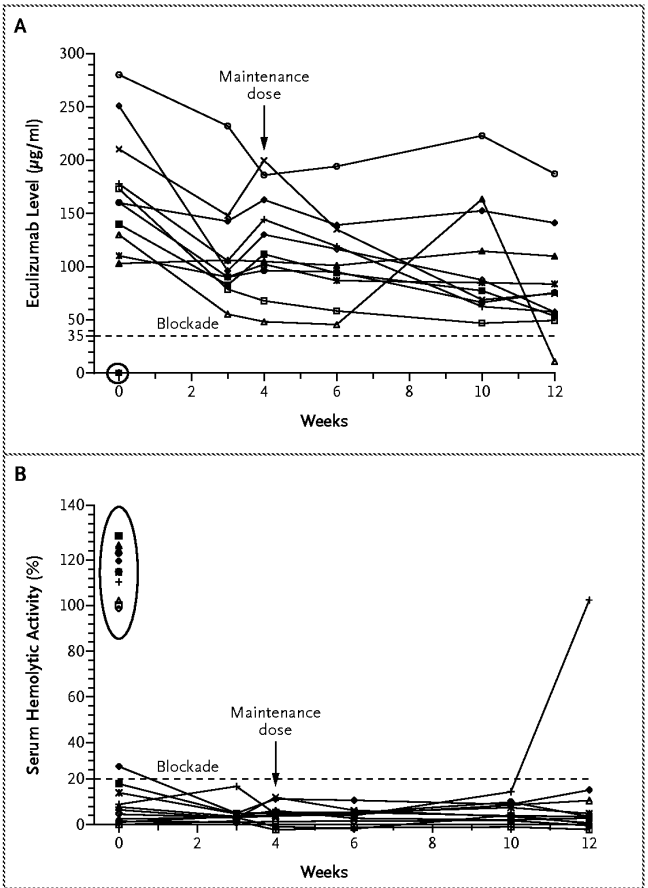

Figure 1. Pharmacokinetic and Pharmacodynamic Analysis of Eculizumab in 11 Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH).

Panel A depicts serum levels of eculizumab during the 12-week treatment period. Eculizumab levels just before a patient's first dose of eculizumab are circled on the x axis. Also shown at time zero are levels of eculizumab one hour after the initial dose. Other data points indicate trough levels of eculizumab for each patient at weeks 3, 4, 6, 10, and 12. The beginning of the 900-mg maintenance dose is indicated by an arrow. The level of eculizumab required to block complement activity (≥35 μg per milliliter) is indicated by a dashed line. Panel B shows hemolytic activity in serum during the 12-week treatment period, as assessed in a presensitized chicken-erythrocyte hemolytic assay. Pretreatment serum hemolytic activity values are circled at time zero. Also shown at time zero are values for hemolytic activity one hour after the initial dose. Other data points indicate trough hemolytic activity at weeks 3, 4, 6, 10, and 12. The dashed line indicates the level of hemolytic activity that is considered to represent complete complement blockade (≤20 percent).

METHODS

PATIENTS

The study was conducted from May through December 2002. Men and women (18 years of age and older) who had received a diagnosis of PNH at least six months earlier, had a detectable GPI-deficient hematopoietic clone, and had received at least four red-cell transfusions in the preceding 12 months were

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eligible. Patients were required to have a negative throat culture for *Neisseria meningitidis* and *N. gonorrhoeae*. All patients were vaccinated against *N. meningitidis* (Mengivac (A+C), Aventis Pasteur) before treatment. One patient had a stroke after consent but never received eculizumab and was excluded. Patients who were taking stable doses of immunosuppressive drugs (e.g., cyclosporine), warfarin, and iron supplements were permitted to continue them.

The trial was approved by the local research ethics committee and was performed according to the International Conference on Harmonisation and Good Clinical Practice Standards. Eleven patients gave written informed consent and were treated with eculizumab.

TREATMENT SCHEDULE

Patients received infusions of 600 mg of eculizumab weekly for four weeks, followed one week later by a 900-mg dose and then by a dose of 900 mg every other week through week 12.

INVESTIGATIONS

In this open-label pilot study, we obtained data on the pharmacokinetics, pharmacodynamics, and immunogenicity of eculizumab and observed its clinical effects by measuring the following: lactate dehydrogenase, haptoglobin, bilirubin, and hemoglobin levels; reticulocyte counts; the proportion of GPI-deficient cells, as assessed by flow cytometry[17]; the rate of transfusion with packed red cells; the rate of occurrence of hemoglobinuria (assessed by daily comparison of the first morning urine sample with a standardized color chart before and during treatment); and the quality of life, as reflected by the scores on the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 instrument. The trigger for transfusion during the study period remained unchanged for each patient, as compared with their care before entry into the study: patients received blood transfusions when they had symptoms resulting from anemia.

Assessment of the safety of eculizumab included ascertainment of treatment-related adverse events, electrocardiography, and routine laboratory tests (e.g., serum chemical analyses and complete blood counts).

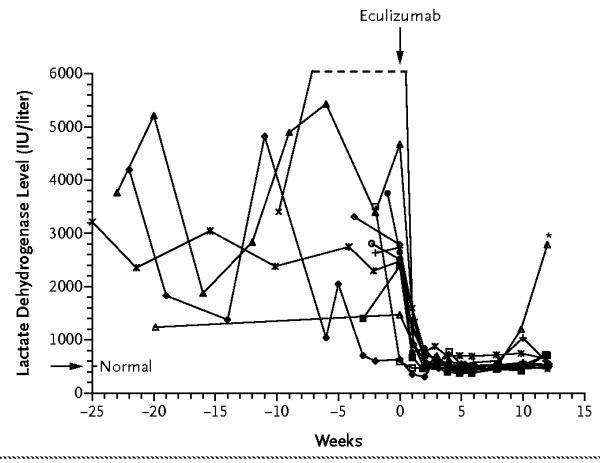

Figure 2. Analysis of Lactate Dehydrogenase Levels, a Biochemical Indicator of Hemolysis, in 11 Patients with Paroxysmal Nocturnal Hemoglobinuria up to 25 Weeks before and during 12 Weeks of Eculizumab Treatment.

The first dose of eculizumab is indicated by an arrow, as is the upper limit of the normal range of lactate dehydrogenase at the Leeds Teaching Hospital. The data point identified at week 12 by the asterisk represents a reading that was obtained from a duplicate serum sample since the original sample was lost. The dashed line represents off-scale points from one patient with a peak value of 12,100 IU per liter.

ASSAY METHODS

The pharmacokinetics of eculizumab were determined with an enzyme-linked immunosorbent assay that detects both free and C5-bound eculizumab.[15] The pharmacodynamics of eculizumab were determined by measuring the capacity of the patient's serum to lyse chicken erythrocytes in a standard total human serum-complement hemolytic assay.[18] The presence or absence of antibodies against eculizumab was assessed by an enzyme-linked immunosorbent assay.[19]

STATISTICAL ANALYSIS

Biochemical values were compared with the use of a paired Student's t-test, quality-of-life measurements with the use of a mixed-effect analysis of covariance, the median rate of transfusions with the use of a Wilcoxon signed-rank test, and the comparison of the number of days with paroxysms with the use of Fisher's exact test.

The corresponding author and the sponsor were jointly responsible for the design of this trial and the development of the protocol. Data were collected and analyzed by a clinical research organization, Kendle International, which maintained the trial data base and provided statistical support. The manuscript was prepared by the corresponding author, with substantial review and comments by the other authors and the sponsor. Final decisions on

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the content of the manuscript rested with the corresponding author in consultation with the other authors. All authors had access to the primary data.

RESULTS

DEMOGRAPHIC CHARACTERISTICS

Six men and five women (median age, 48 years; range, 21 to 67) with a median duration of PNH of 8.6 years (range, 1.7 to 37.4) participated in the trial. Five of the patients had platelet counts at base line of less than 150,000 per cubic millimeter. Eight patients had previously received a diagnosis of aplastic anemia, two were concomitantly receiving cyclosporine for aplastic anemia, and six were receiving warfarin.

SAFETY

All patients completed the 12-week study. There were no deaths or thrombotic events, and all patients subsequently entered a 12-month extension study. Each patient reported one or more adverse events during the trial. Events reported by three patients included headache and upper respiratory tract infection. Events reported by two patients included influenza-like symptoms, rigors, dizziness, nausea, nasal congestion, and joint aches. None of these events were attributed to the study medication. Serious adverse events occurred in two patients. The first was hospitalized with a viral chest infection. The second reported nausea, vomiting, and headache after the first infusion, with dizziness and shivering the following day. The patient was hospitalized overnight, and subsequent infusions were well tolerated.

PHARMACOKINETICS, PHARMACODYNAMICS, AND IMMUNOGENICITY OF ECULIZUMAB

Peak and trough levels of eculizumab were well above 35 μg per milliliter from one hour after the first dose through the completion of the 600-mg weekly dose period (Fig. 1A). In 10 patients, serum trough levels of eculizumab remained above 35 μg per milliliter for the entire study.

The hemolytic activity of serum from these 10 patients was completely blocked (less than 20 percent in the chicken–red-cell assay) for essentially the entire treatment period (Fig. 1B). In 1 of the 11 patients, the trough level of eculizumab fell below 35 μg per milliliter at week 12 and serum hemolytic activity returned. In no case were antibodies against eculizumab detected.

BIOCHEMICAL INDICATORS OF HEMOLYSIS

Levels of lactate dehydrogenase in serum were markedly elevated in all patients before eculizumab treatment. Mean (±SD) lactate dehydrogenase levels fell from 3111±598 IU per liter during the 12 months before enrollment to 594±32 IU per liter (normal range, 150 to 480) during treatment ($P=0.002$) (Fig. 2).

The decrease in lactate dehydrogenase began after a single dose of eculizumab in all patients. Lactate dehydrogenase levels remained within or just above the normal range for the duration of the study (Fig. 2). In the one patient in whom eculizumab levels fell below 35 μg per milliliter at week 12 (Fig. 1A), hemolytic activity returned (Fig. 1B) and lactate dehydrogenase levels increased transiently (Fig. 2).

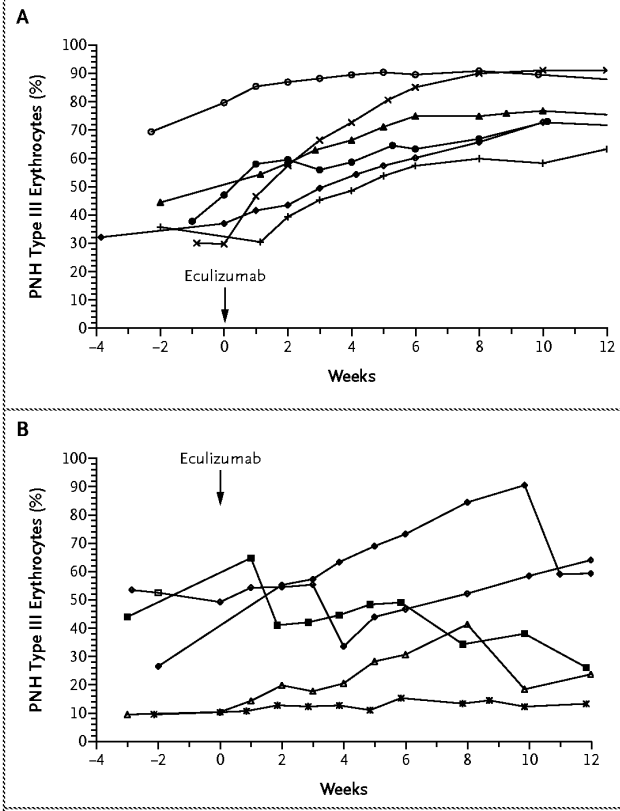

Figure 3. Changes in the Percentage of Paroxysmal Nocturnal Hemoglobinuria (PNH) Type III Erythrocytes during Eculizumab Treatment in Six Patients Who Received No Transfusions during Treatment (Panel A) and Five Patients Who Received at Least One Transfusion after Starting Treatment (Panel B).
The initial dose of eculizumab is indicated by the arrow in each panel.

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The dosing frequency was increased from 900 mg every 14 days to 900 mg every 12 days, reestablishing complete complement blockade during the ongoing 12-month extension study (data not shown).

Haptoglobin became detectable in the serum of 5 of the 11 patients after two weeks of eculizumab treatment but returned to undetectable levels soon thereafter (data not shown). Bilirubin levels were also elevated in most patients at base line and did not change significantly during treatment (data not shown).

EFFECT ON PNH CLONES

Type III erythrocytes are highly sensitive to lysis by complement and as a result have a short life span. In our study, the percentage of type III erythrocytes increased significantly from a mean of 36.7±5.9 percent before treatment to 59.2±8.0 percent at the end of 12 weeks of treatment (P=0.005) (Fig. 3). The increase was particularly consistent in six patients who remained transfusion-independent during the study (Fig. 3A). In patients who received transfusions during the study, sudden drops in the proportion of type III cells were seen as the transfused red cells diluted the type III population (Fig. 3B). There were no significant changes in the percentages of PNH type III neutrophils, monocytes, or platelets during treatment with eculizumab; in most cases, these percentages were 90 to 100 percent before the study (data not shown).

TRANSFUSION REQUIREMENTS, HEMOGLOBIN LEVELS, AND RETICULOCYTE COUNTS

During the year preceding enrollment, the range of red-cell transfusions received by the 11 patients was 12 to 55 units, whereas during the three months of the study, the range was 0 to 8 units. Before eculizumab treatment, the mean and median transfusion rates were 2.1 and 1.8 units per patient per month, respectively (Table 1). These transfusion rates decreased to 0.6 and 0.0 unit per patient per month, respectively, during the three months of treatment with eculizumab (P=0.003). Hemoglobin levels did not increase significantly during the treatment period, although hemoglobin values in six patients stabilized without transfusions (Table 1). Similarly, the numbers of reticulocytes remained relatively constant during eculizumab treatment.

HEMOGLOBINURIA

Our patients recorded the color of their urine each morning using a color chart designed to assess the

Table 1. **Transfusion Rates, Hemoglobin Levels, and Reticulocyte Counts 12 Months before and after 3 Months of Eculizumab Treatment.**

Patient No.	12 Mo before Eculizumab Treatment				After 3 Mo of Eculizumab Treatment			
	Transfusions		Hemoglobin	Reticulocytes	Transfusions		Hemoglobin	Reticulocytes
	no. of units	*rate**	*g/dl*	*×10⁻³/mm³*	*no. of units*	*rate†*	*g/dl*	*×10⁻³/mm³*
1	22	1.8	10.3	77.5	2	0.7	10.0	100.7
2	23	1.9	8.3	200.0	8	2.9	8.8	182.6
3	20	1.6	10.1	169.5	0	0.0	10.7	175.9
4	28	2.3	9.3	282.0	0	0.0	9.4	333.3
5	12	1.0	11.9	96.3	2	0.7	10.6	121.8
6	14	1.2	9.8	346.8	0	0.0	10.6	259.0
7	34	2.8	12.8	100.6	0	0.0	13.5	166.8
8	21	1.7	9.5	164.5	0	0.0	9.8	239.6
9	55	4.5	10.7	138.0	3	1.1	11.4	285.8
10	41	3.4	8.5	108.7	5	1.8	8.8	140.1
11	14	1.2	8.5	91.4	0	0.0	10.0	97.4
Median‡		1.8				0.0		
Mean		2.1	10.0	161.4		0.6	10.3	191.2

* The rate (in units per month) was calculated as (number of units÷365 days)×30.
† The rate (in units per month) was calculated as (number of units÷84 days)×30.
‡ P=0.003 for the change in the median rate of transfusion by the Wilcoxon signed-rank test.

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degree of hemoglobinuria (Fig. 4A) during both the 2-to-4-week screening period and the 12-week treatment period. Paroxysms of hemoglobinuria were prospectively defined as dark-colored urine with a colorimetric level of 6 or more. In nine patients for whom urine scores were assessed, the mean incidence of paroxysms was reduced from 2.9 days to 0.12 day per patient per month ($P<0.001$) (Fig. 4B).

QUALITY OF LIFE

The quality of life was assessed with the use of the EORTC QLQ-C30 instrument. When responses at base line were compared with responses during 12 weeks of eculizumab treatment, there were significant improvements in the domains of global health status ($P=0.02$), physical functioning ($P<0.001$), emotional functioning ($P<0.001$), cognitive functioning ($P=0.002$), fatigue ($P<0.001$), dyspnea ($P=0.002$), and insomnia ($P=0.049$) (Table 2).

DISCUSSION

Patients with PNH have chronic, often disabling symptoms of fatigue and intermittent episodes of dysphagia, abdominal pain, and hemoglobinuria. These symptoms are thought to be related to the intravascular destruction of PNH type III erythrocytes, which are deficient in complement inhibitors, by autologous complement. The hemolytic anemia frequently renders the patients transfusion-dependent. In addition, patients have an extremely high risk of potentially life-threatening thrombosis, particularly thrombosis of the hepatic and cerebral veins. Approximately 50 percent of patients with PNH die of the disease; the median duration of survival after diagnosis is 10 years.[1]

We found that the defect in the membrane-bound inhibitor of terminal complement components in PNH was ameliorated by the administration of eculizumab. This antibody specifically prevents cleavage of C5, which is necessary for assembly of the membrane-attack complex. Blockade of terminal complement components presumably prolongs the survival of type III erythrocytes (since there was no simultaneous increase in reticulocytes), which are highly sensitive to lysis by complement, thereby increasing the proportion of these cells in the blood and reducing signs of hemolysis in most patients. In some patients, the percentage of type III erythrocytes increased to more than 80 percent of the total erythrocyte population. This interpretation of the mechanism of action of eculizumab is consistent with the report of an asymptomatic patient with PNH who had more than 80 percent type III erythrocytes and a concomitant deficiency in the terminal complement protein C9.[20] Thus, inhibition of the assembly of C5b–C9 by an antibody or by a congenital deficiency of a terminal component of the complement system can protect type III erythrocytes from complement-mediated lysis.

The long-term effects of protecting PNH type III erythrocytes from complement are not known. For example, will removing the negative pressure

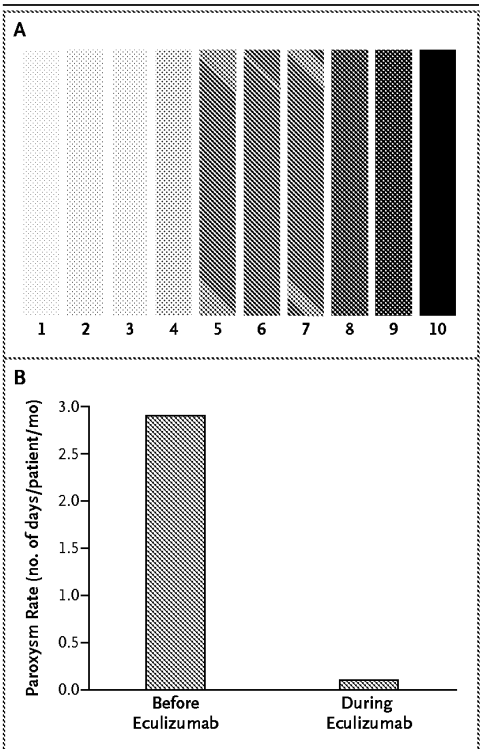

Figure 4. **Incidence of Paroxysms during Eculizumab Treatment.**

Panel A shows a urine color scale devised to monitor the incidence of paroxysms of hemoglobinuria in patients with paroxysmal nocturnal hemoglobinuria before and during treatment. A paroxysm was prospectively defined as a urine score of 6 or greater in this study. Panel B depicts the change in the paroxysm rate (defined as the mean number of days in paroxysm per patient per month) in nine patients 1 month before and during 12 weeks of eculizumab therapy. Pretreatment data on paroxysms were inadvertently not collected for two patients, and these two were therefore excluded from the analysis.

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Table 2. **Change in the Quality of Life during Eculizumab Treatment.***

Domain	Mean Base-Line Score†	Change from Base Line‡§	P Value§
Global health status	56.1	13.7	0.02
Physical functioning	70.9	13.0	<0.001
Emotional functioning	70.5	12.7	<0.001
Cognitive functioning	77.3	11.8	0.002
Fatigue	47.5	−15.3	<0.001
Dyspnea	39.4	−12.4	0.002
Insomnia	30.3	−10.8	0.049

* The quality of life was assessed with the European Organization for Research and Treatment of Cancer QLQ-C30 instrument.
† Numbers represent mean values of linearly transformed scores.
‡ Values for change from base line represent least-square means. A positive value indicates an improvement in the score for global health status, physical functioning, emotional functioning, and cognitive functioning, whereas a negative value indicates an improvement in the score for fatigue, dyspnea, and insomnia.
§ Values are from a mixed analysis-of-covariance model with visit as a fixed effect, patient as a random effect, and base line as a covariate.

on type III hematopoietic cells alter the rate of expansion of the PNH clone? What might occur in a patient with an increased population of PNH type III erythrocytes if treatment with eculizumab is stopped? Two of the patients who entered the eculizumab extension study had transient breakthroughs (lasting two to three days) in complement blockade until the dosing interval was adjusted. Both patients had hemoglobinuria with mild symptoms, but the episodes were not life threatening and were easily managed (data not shown). Definitive answers to these questions will require further study.

In this trial, lactate dehydrogenase levels declined rapidly and remained reduced as long as the serum level of eculizumab exceeded 35 μg per milliliter. The importance of maintaining this level of antibody was demonstrated in a single patient, in whom the eculizumab level transiently dropped below 35 μg per milliliter at week 12, resulting in a return of serum complement activity and an increase in lactate dehydrogenase levels. Subsequent administration of eculizumab reestablished complement blockade and rapidly reduced lactate dehydrogenase levels. Interestingly, lactate dehydrogenase levels were reduced in most patients to just above the upper limit of normal. The slightly elevated levels of this enzyme during treatment with eculizumab could reflect persistent, low-level C3b-mediated extravascular hemolysis or, possibly, undefined mechanisms of hemolysis that are unrelated to complement.

We also found that eculizumab treatment significantly reduced transfusion requirements, even though the levels of hemoglobin did not change significantly. However, the hemoglobin level in an individual patient before study entry was artificially maintained as a result of the transfusion of normal red cells. The transfused red cells survive far longer than PNH cells. Therefore, the stabilization of hemoglobin levels with a reduced need or no need for transfusion is a result of the protection of PNH red cells from complement-mediated lysis by eculizumab.

The decrease in transfusion requirements was most apparent in the six patients without a clinically significant degree of bone marrow failure (as defined by a normal platelet count). All but one of these patients were transfusion-independent during the study and remained so during an extension study. The remaining patient received a single 3-unit transfusion during the study, as compared with the receipt of 55 units in the 12 months preceding enrollment.

There was a rapid improvement in the quality of life during eculizumab therapy, as measured by the EORTC QLQ-C30. These clinical observations support the hypothesis that many of the important coexisting clinical conditions in patients with PNH are directly related to chronic and acute episodes of hemolysis, possibly through the scavenging of nitric oxide by plasma free hemoglobin.[21-25]

Eculizumab was safe and well tolerated during this open-label pilot study. The adverse events reported by patients were similar in type and frequency to those reported with either eculizumab or placebo in other controlled trials. All patients are currently participating in a one-year extension study in which the drug continues to be well tolerated. Furthermore, the rates of intravascular hemolysis, as measured by lactate dehydrogenase levels and hemoglobinuria, remain reduced in all patients, with 5 of 11 patients having been transfusion-independent for at least one year since starting eculizumab treatment.

The *PIG-A* mutation in patients with PNH causes deficiencies in the membrane-bound complement inhibitors CD55 and CD59, resulting in intravascular hemolysis.[2,3] However, patients who have genetic deficiencies in the surface expression of CD55 (Inab phenotype) with normal levels of CD59 have no clinical signs of hemolysis.[8,9] Conversely, a pa-

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tient with a genetic deficiency in the expression of CD59 but normal levels of CD55 had symptoms indistinguishable from those of PNH.[10,11] Therefore, the somatic mutation in the *PIG-A* gene that causes the deficiency of the membrane-bound terminal complement inhibitor CD59 is critical to the pathogenesis of PNH. We found that terminal complement inhibition with eculizumab ameliorated the untoward effects of this deficiency.

In summary, eculizumab appears to enhance the survival of type III PNH erythrocytes, improving the quality of life and reducing the extent of hemolysis, hemoglobinuria (the clinical hallmark of PNH), and the need for blood transfusions in patients with PNH. This study confirms that terminal complement activation is the key mediator of erythrocyte destruction in PNH.

Funded by Alexion Pharmaceuticals, Cheshire, Conn.

Dr. Hillmen reports serving as a consultant to Alexion Pharmaceuticals and receiving grant support from the company; Drs. Rollins, Mojcik, and Rother, Mr. Bombara, and Ms. Petro report having equity ownership in Alexion Pharmaceuticals; and Drs. Rollins and Rother have assigned to Alexion Pharmaceuticals their inventions made as employees of the company and have received no royalties from Alexion for these inventions. Dr. Rollins receives royalties for inventions he made before becoming an employee of Alexion.

We are indebted to Drs. Leonard Bell, Stephen Squinto, and Paul Finnegan for critical review of the manuscript; to Karen Hannon for excellent technical support; and to Jan Farish, David Buchanon, Pilar Hernandez-Campo, and the nurses of Healthcare-at-Home for their assistance in carrying out the study.

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Report on the Deliberation Results

March 5, 2010

Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau
Ministry of Health, Labour and Welfare

[Brand name]	Soliris for Intravenous Infusion 300 mg
[Non-proprietary name]	Eculizumab (Genetical Recombination) (JAN*)
[Applicant]	Alexion Pharma K.K.
[Date of application]	March 31, 2009

[Results of deliberation]

In the meeting held on February 26, 2010, the First Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product is classified as a biological product, the re-examination period is 10 years, and the drug substance and the drug product are both classified as powerful drugs.

[Conditions for approval]

1. Due to limited data from Japanese clinical studies, conduct a post-marketing drug use-results survey over a certain period of time, covering all patients treated with Soliris, in order to obtain the background information of patients treated with Soliris, collect data on the safety and efficacy of Soliris, and take necessary measures to ensure proper use of Soliris.
2. Prior to marketing Soliris, take necessary measures to ensure that Soliris will be administered only under the supervision of a physician at a medical institution, who is familiar with the diagnosis and treatment of paroxysmal nocturnal hemoglobinuria and is also fully capable of managing the risks etc. associated with Soliris, in cooperation with a physician who is familiar with the diagnosis and treatment of meningococcal infection.

**Japanese Accepted Name (modified INN)*

This English version of the Japanese review report is intended to be a reference material to provide convenience for users. In the event of inconsistency between the Japanese original and this English translation, the former shall prevail. The PMDA will not be responsible for any consequence resulting from the use of this English version.

Review Report

February 18, 2010
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Soliris for Intravenous Infusion 300 mg (Note: The proposed Japanese brand name has been modified.)
[Non-proprietary name]	Eculizumab (Genetical Recombination)
[Name of applicant]	Alexion Pharma K.K.
[Date of application]	March 31, 2009
[Dosage form/Strength]	Concentrated solution for intravenous infusion: Each vial of 30 mL contains 300 mg of Eculizumab (Genetical Recombination).
[Application classification]	Prescription drug (1) Drug with a new active ingredient

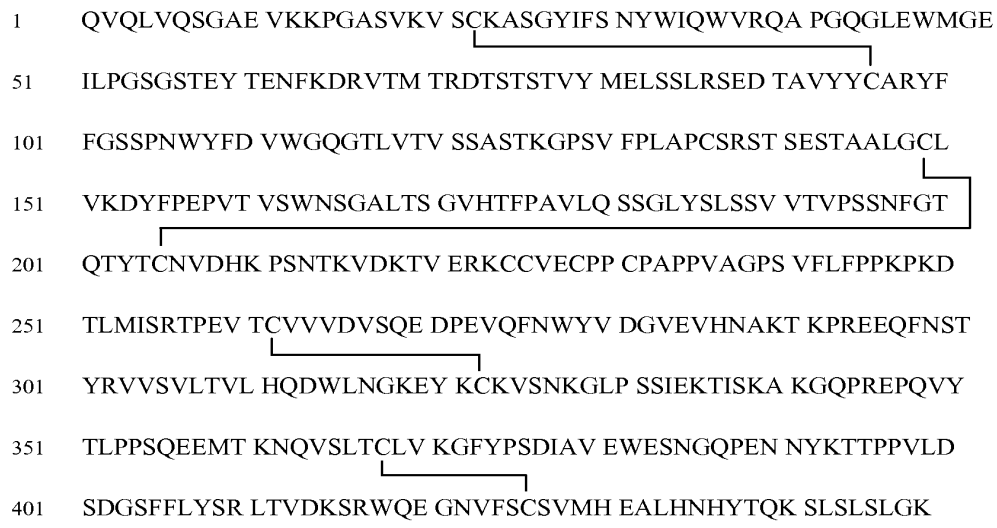
[Entity]

Eculizumab is a recombinant humanized monoclonal antibody composed of the variable regions consisting of complementarity-determining regions derived from mouse anti-human complement C5α-chain monoclonal antibody and human framework regions and human IgG constant regions. The L-chain constant region is derived from a human κ-chain. The CH1, hinge and a part of CH2 regions of the H-chain constant regions are derived from human IgG2 (γ2-chain) and the rest of CH2 and CH3 regions are derived from human IgG4 (γ4-chain).
Eculizumab is produced in mouse myeloma cell (NS0).
Eculizumab is a glycoprotein (molecular weight, ca.145,235) composed of 2 H-chain molecules consisting of 448 amino acid residues each and 2 L-chain molecules consisting of 214 amino acid residues each.

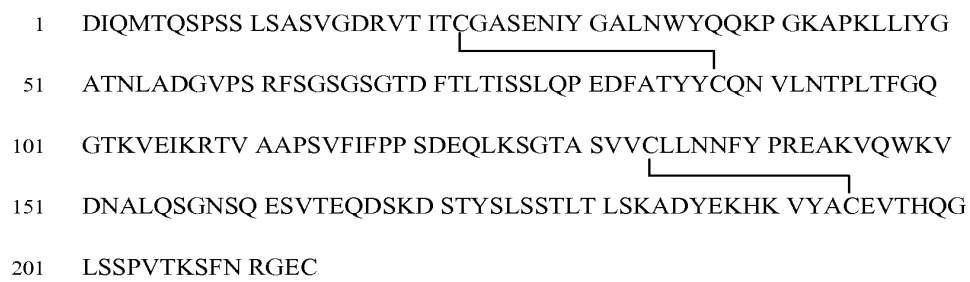
This English version of the Japanese review report is intended to be a reference material to provide convenience for users. In the event of inconsistency between the Japanese original and this English translation, the former shall prevail. The PMDA will not be responsible for any consequence resulting from the use of this English version.

Structural formulae:

H-chain



L-chain



[The amino acid sequence of Eculizumab (Genetical Recombination)]

pyroglutamic acid at Q1 of the H-chain; glycosylation at N298 of the H-chain; partial processing at K448 of the H-chain; inter-chain disulfide bonds of C136 of the H-chain–C214 of the L-chain, C224 of the H-chain–C224 of the H-chain, C225 of the H-chain–C225 of the H-chain, C228 of the H-chain–C228 of the H-chain, and C231 of the H-chain–C231 of the H-chain (intra-chain disulfide bonds are represented by solid lines); murine complementarity-determining regions (CDRs): N31 to Q35 of the H-chain, E50 to D66 of the H-chain, Y99 to V111 of the H-chain, G24 to N34 of the L-chain, G50 to D56 of the L-chain, and Q89 to T97 of the L-chain

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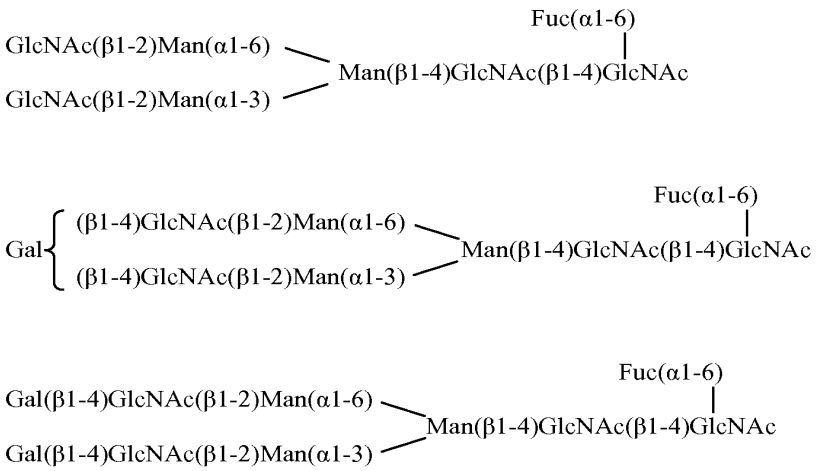

[Predicted main carbohydrate structures of Eculizumab (Genetical Recombination)]

[Items warranting special mention]	Orphan drug ("Orphan Drug Designation" [PFSB/ELD Notification No. 1222007 dated December 22, 2008])
[Reviewing office]	Office of New Drug I

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Review Results

February 18, 2010

[Brand name]	Soliris for Intravenous Infusion 300mg (Note: The proposed Japanese brand name has been modified.)
[Non-proprietary name]	Eculizumab (Genetical Recombination)
[Name of applicant]	Alexion Pharma K.K.
[Date of application]	March 31, 2009

[Results of review]

Based on the submitted data, the efficacy of the product in patients with paroxysmal nocturnal hemoglobinuria has been demonstrated. On the other hand, regarding safety, as the use of eculizumab may increase the risk of developing a meningococcal infection and a serious hemolytic crisis may occur after eculizumab discontinuation, it is necessary to inform physicians and patients of these risks and take adequate safety measures. The safety of the product is acceptable in view of its observed benefits, provided that the product is used under a proper safety control system.

As a result of its regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product may be approved for the indication and dosage and administration as shown below, with the following conditions.

[Indication]

Reduction of hemolysis in patients with paroxysmal nocturnal hemoglobinuria

[Dosage and administration]

The usual adult dosage is 600 mg as Eculizumab (Genetical Recombination) given as an intravenous infusion weekly for a total of 4 doses, followed 1 week later (4 weeks after the first dose) by 900 mg given every 2 weeks thereafter.

[Conditions for approval]

1. Due to limited data from Japanese clinical studies, conduct a post-marketing drug use-results survey over a certain period of time, covering all patients treated with Soliris, in order to obtain the background information of patients treated with Soliris, collect data on the safety and efficacy of Soliris, and take necessary measures to ensure proper use of Soliris.
2. Prior to marketing Soliris, take necessary measures to ensure that Soliris will be administered only under the supervision of a physician at a medical institution, who is familiar with the diagnosis and treatment of paroxysmal nocturnal hemoglobinuria and is also fully capable of managing the risks etc. associated with Soliris, in cooperation with a physician who is familiar with the diagnosis and treatment of meningococcal infection.

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Review Report (1)

December 16, 2009

I. Product Submitted for Registration

[Brand name]	Soliris for Intravenous Infusion 300 mg
[Non-proprietary name]	Eculizumab (Genetical Recombination)
[Name of applicant]	Alexion Pharma K.K.
[Date of application]	March 31, 2009
[Dosage form/Strength]	Concentrated solution for intravenous infusion: Each vial of 30 mL contains 300 mg of Eculizumab (Genetical Recombination).
[Proposed indication]	Paroxysmal nocturnal hemoglobinuria (PNH)
[Proposed dosage and administration]	Soliris should be administered by intravenous infusion at a dose of 600 mg every 7 days for the first 4 weeks followed by 900 mg for the fifth dose 7 days later and then 900 mg every 14 days thereafter.

II. Summary of the Submitted Data and Outline of Review by the Pharmaceuticals and Medical Devices Agency

A summary of the submitted data and an outline of the review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below.

1. Origin or history of discovery and usage conditions in foreign countries etc.

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired genetic disorder due to a somatic mutation of the phosphatidylinositol glycan-A (PIG-A) gene on chromosome X, which is necessary for the biosynthesis of glycosylphosphatidylinositol (GPI) anchors. The mutation leads to a deficiency of the GPI-anchored terminal complement inhibitor CD59 on the surface of clonal populations of hematopoietic stem cells. CD59 inhibits C9 binding to complement C5 and blocks the formation of the terminal complement complex C5b-9 on the red blood cell (RBC) surface. Deposition of cytolytic C5b-9 on the surface of CD59-deficient PNH RBCs results in intravascular hemolysis. PNH is characterized by recurrent episodes of hemolysis and chronic hemolysis, and intravascular hemolysis leads to hemoglobinuria and fatigue and poor physical functioning associated with anemia. It is thought that intravascular hemolysis releases excessive amount of hemoglobin (Hb), which induces the activation of platelets and blood coagulation cascade, causing thrombosis. Meanwhile, free Hb deposition in the kidney may lead to kidney diseases such as nephritis and renal tubule abnormalities.

According to the results of a 1998 survey conducted by a major research institution, the estimated

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number of PNH patients in Japan is approximately 430 (Health Science Research Grants, Research Project on Intractable Diseases, Research Committee for determining the epidemiology of diseases not covered by Research Project on Treatment of Intractable Diseases, 1999 Research Results, 2000). A comparative survey between Japan and the US revealed that in Japan, the median survival time after diagnosis is 25.0 years and the main causes of death are thrombosis, haemorrhage, infections, and renal failure (*Medicine*. 2004;83:193-207.).

There are only palliative therapies for PNH that aim to relieve symptoms temporarily. Blood transfusions are used for temporarily improving the symptoms associated with anemia, short-term steroid therapy is used for acute hemolysis, and anticoagulant therapy is used to prevent and treat thrombosis. Although allogeneic hematopoietic stem cell transplantation is available as a curative option, as PNH is a slowly progressive disease, it is indicated for severe patients only, taking account of the risks associated with transplantation.

Eculizumab (Genetical Recombination) (eculizumab) is a humanized monoclonal antibody that binds with high affinity to human complement C5. Eculizumab is expected to reduce intravascular hemolysis mediated by C5b-9 by inhibiting C5 activity and the development of eculizumab for PNH patients was initiated.

Eculizumab received approval in the US in March 2007. As of October 2009, eculizumab has been approved for the indication of PNH in 33 countries including the US and Europe.

Eculizumab was designated as an orphan drug for the intended indication of "paroxysmal nocturnal hemoglobinuria" as of December 22, 2008.

2. Data relating to quality

2.A Summary of the submitted data

Eculizumab is a glycoprotein (approximately 145,235 Da) composed of two 448 amino acid heavy chains ($C_{2205}H_{3378}N_{576}O_{683}S_{19}$, approximately 49,493 Da) and two 214 amino acid light chains ($C_{1016}H_{1583}N_{271}O_{334}S_{6}$, approximately 23,130 Da) produced in a murine myeloma cell line (NS0 cell line). The molecule contains 14 disulfide bonds and there is one *N*-linked glycosylation site on the C_H2 region of the heavy chain.

Eculizumab is a humanized anti-human C5 monoclonal antibody composed of the variable regions consisting of complementarity determining regions (CDRs) derived from mouse anti-human C5α chain monoclonal antibody (m5G1.1 mAb) and human framework regions, and human IgG constant regions. The C_H1, hinge and a part of C_H2 regions of the heavy chain constant regions are derived from human IgG_2 (γ2-chain) and the rest of C_H2 and C_H3 regions are derived from human IgG_4 (γ4-chain). The light

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chain constant region is derived from a human κ-chain.

2.A.(1) Drug substance

2.A.(1).1) Manufacturing process

(a) Assembly of the expression construct and cell banking

To generate and select m5G1.1 mAb, hybridomas were generated from mice immunized with human complement component C5 and the antibodies produced by these hybridomas were evaluated for their ability to block complement-mediated hemolysis and C5a generation. The m5G1.1 mAb gene was cloned from cDNA prepared from the hybridoma. The CDRs of the genes for human heavy chain variable and C_H1 regions (hereinafter referred to as "Fd fragment") and for a human light chain were replaced by the CDRs from the genes for the variable heavy and light chains of the m5G1.1 mAb, respectively. Thus, an intermediate vector containing the gene encoding a heavy chain Fd fragment ("[redacted] + [redacted]") and an intermediate vector containing the gene encoding a light chain ("[redacted] + [redacted]") were constructed. Then, the DNA segment encoding the heavy chain formed by joining the DNA fragment encoding the heavy chain Fd fragment excised from [redacted] with a DNA fragment encoding the hinge, C_H2, and C_H3 regions, and the DNA segment encoding the light chain excised from [redacted] were inserted to construct a double-gene vector ([redacted]). The DNA segments encoding the heavy and light chains excised from the vector were inserted into a vector containing the glutamine synthetase gene, which functions as a selection marker, to generate the expression plasmid [redacted].

[redacted] was introduced by electroporation into a NS0 cell line. The cell clones selected in serum-supplemented medium containing [redacted] were adapted to serum-free medium, and pre-seed stock [redacted] of the seed cell line [redacted] was prepared. This [redacted] was cultured in the medium for preparing cell banks ([redacted]) to prepare a master cell bank (MCB) [redacted]M and a working cell bank (WCB) was prepared from [redacted]M.

(b) Characterization and control of cell banks

The results of tests for identity and purity of the MCB, WCB, and cells at the limit of *in vitro* cell age used for production (CAL) are shown in Table 1. The results of genetic stability studies on the MCB, WCB, end of production cells (EOP), and CAL are shown in Table 2.

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Table 1. Results of tests for identity and purity of cell banks and CAL

Test			Results		
			MCB	WCB	CAL
			[redacted]M	[redacted]W	[redacted] (Process [redacted])
Cell viability			Constant		NT
Isoenzyme analysis			Of mouse origin		
Mycoplasma testing (culture method, indicator cell culture method)			Negative		
Sterility test (bacteria/fungi)			No evidence of microbial growth 14 days later		NT
Tests for adventitious viruses	*In vitro* assay for adventitious viruses		Negative (Balb-C, HeLa, MRC-5, NS0, RK-13, Vero)	Negative (MRC-5, NS0, Vero)	Negative (MRC-5, NIH/3T3, Vero)
	In vitro bovine virus assay		Negative (BT, MA104)	Negative (BT, Vero)	NT
	In vivo assay to reveal latent viruses (inoculation into suckling and adult mice and guinea pigs, and in embryonated eggs)		Negative	NT	Negative
	Mouse antibody production test*		Negative	NT	Negative
	Quantitative PCR		Negative (bovine polyoma virus, bovine and porcine circovirus, MVM)	NT	Negative (MVM)
Tests for retroviruses	Transmission electron microscopy	Examination of cell sections	A-type and C-type retrovirus-like particles were observed.	NT	A-type and C-type retrovirus-like particles were observed.
		Negative staining	NT	NT	Retrovirus-like particle count < 1.4×10^5/mL
	Mink S^+L^- focus assay		Direct assay: 27 ffu/mL Extended assay: fusion foci	NT	Negative
	In vitro XC plaque assay		Negative	NT	Negative
	In vitro assay for infectious X MuLV		Negative	NT	Negative
	Reverse transcriptase activity (Mg^{2+} and Mn^{2+} dependent)		Negative	NT	Negative
	Cocultivation (MRC-5, LEX98)		Negative	NT	Negative

NT: No Tested

Balb-C: BALB/c mouse embryo cell line, BT: bovine nasal turbinate cell line, HeLa: human cervical carcinoma cell line, MA104: fetal rhesus monkey kidney cell line, MRC-5: human diploid lung cell line, MVM: minute virus of mice, NIH/3T3: mouse fibroblast cell line, RK-13: rabbit kidney cell line, Vero: African green monkey kidney cell line, X MuLV: xenotropic murine leukemia virus

*Lymphocytic Choriomeningitis Virus, Mouse Hepatitis Virus, Pneumonia Virus of Mice, Minute Virus of Mice, Sendai Virus, Ectromelia Virus, Mouse Rotavirus, Reovirus Type 3, Mouse Encephalomyelitis Virus, Mouse Adenovirus, Polyoma Virus, Hantaan Virus, Mouse Thymic Virus, Mouse Salivary Gland Virus, K Virus, Lactic Dehydrogenase Virus

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Table 2. Results of genetic stability studies on cell banks, EOP, and CAL

Test item (Test method)		Results				
		MCB	WCB	EOP		CAL
		[redacted]M	[redacted]W	[redacted] (Process [redacted])	[redacted] (Process [redacted])	[redacted] (Process [redacted])
Site of integration of expression plasmid (Southern blotting)		Expected bands were detected.	Identical banding pattern with MCB			
Copy number/cell (Southern blotting)	Heavy chain	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Light chain	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Heavy and light chain mRNA (Northern blotting)		Expected bands were detected.	NT			Identical banding pattern with MCB
cDNA sequence		Identical to the nucleic acid sequence determined for the expression construct	NT			Identical to the nucleic acid sequence determined for the expression construct

NT: No Tested

The MCB and WCB are preserved at -100°C at [redacted] sites and it has been confirmed that they are stable for [redacted] years. Whether the shelf life may be extended will be determined by assessing cell viability and cellular proliferative capacity every [redacted] years.

An additional WCB will be prepared in accordance with the current cell banking procedures based on the number of ampoules left and a newly prepared WCB will be qualified (identity [cell viability, isoenzyme analysis], purity [sterility test, mycoplasma testing, *in vitro* assay for adventitious viruses (MRC-5, NIH/3T3, Vero)]).

There is no plan for generating a new MCB.

(c) Manufacturing process

The manufacturing process and controls for the drug substance are as shown below.

Manufacturing process		Control item
Step 1	Cell thawing and inoculum preparation Cell thawing: [redacted] ampoule from WCB Medium: [redacted]	At thawing: cell [redacted]
Seed culture 1	Equipment: [redacted]mL, [redacted]mL, [redacted]mL flask ([redacted]rpm)	
Seed culture 2-5	Equipment: [redacted]mL, [redacted] mL roller bottle ([redacted]rpm)	
Seed culture 6	Equipment: [redacted]mL spinner flask ([redacted]rpm)	After Step 1 culture: [redacted] cell [redacted], cell [redacted], cell [redacted], bioburden
Step 2a	Expansion of the culture Equipment: [redacted]L bioreactor Medium: [redacted]	At Step 2a inoculation: pH, bioburden, [redacted]
Step 2b	Expansion of the culture Equipment: [redacted]L bioreactor Medium: [redacted]	During Step 2b culture: pH, bioburden, [redacted] After Step 2b culture: cell [redacted]
Step 2c	Production culture Equipment: [redacted]L bioreactor Medium: [redacted]	During Step 2c culture: pH, bioburden, [redacted] [redacted]: mycoplasma testing, test for absence of MVM[a] (quantitative PCR), *in vitro* assay for adventitious viruses (MRC-5, NIH/3T3, Vero)
Step 3	Harvest and concentration Equipment: centrifuge, depth filter ([redacted] [redacted] [redacted] [redacted]µm), [redacted]L container, ultrafiltration membrane ([redacted]kDa)	When the volume of harvest supernatant reaches [redacted] L: bioburden After concentration: bioburden

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Step 4	**Buffer exchange** **Equipment: depth filter ([redacted]µm), hydrophilic membrane filter ([redacted] µm)**	**After filtration: bioburden** **After storage: bioburden**
Step 5	**Affinity chromatography** **Equipment: [redacted][b] protein A [redacted] column**	**At the time of loading: pH, bioburden** **After the first cycle: [redacted]**
Step 6	**Virus inactivation** **Equipment: storage container** **Conditions: pH[redacted], [redacted] minutes**	**During treatment: pH** **After treatment: Step [redacted], pH, bioburden, endotoxin** **Before storage: bioburden, endotoxin**
Step 7	**Concentration/buffer exchange** **Equipment: ultrafiltration membrane ([redacted] kDa)**	**Before concentration: pH, electrical conductivity** **After dialysis: pH, electrical conductivity** **Before concentration adjustment: protein concentration** **After concentration adjustment: bioburden, endotoxin** **Before storage: protein concentration, pH, electrical conductivity**
Step 8	**[redacted]ion exchange chromatography (non-adsorbed)** **Equipment: [redacted] column** **Recovery: non-adsorbed fraction**	**At the time of loading: pH, electrical conductivity, bioburden, endotoxin** **Non-adsorbed fraction: [redacted], pH, electrical conductivity, bioburden, endotoxin**
Step 9	**Virus removal** **Equipment: [redacted] ([redacted]nm)** **Pressure: [redacted]psig ([redacted]bar)**	**After filtration: filter integrity test, bioburden, endotoxin**
Step 10	**[redacted]ion exchange chromatography (bind and elute)** **Equipment: [redacted] column** **Recovery: eluate fraction**	**Eluate before loading: pH, electrical conductivity, endotoxin** **At the time of loading: bioburden, endotoxin** **Eluate: bioburden, endotoxin, pooling criteria (monomer content)**
Step 11	**Preparation of drug substance (concentration/buffer exchange, addition of polysorbate 80)** **Equipment: ultrafiltration membrane ([redacted] kDa)**	**Before concentration: pH, electrical conductivity** **After concentration: protein concentration** **Before permeation: pH, electrical conductivity** **After permeation: protein concentration, bioburden** **Before concentration adjustment: protein concentration** **After addition of polysorbate 80: protein concentration**
Step 12a	**Final sterile filtration and filling** **Equipment: [redacted]L LDPE[c] bag with hydrophilic membrane filter ([redacted] µm)**	**After filtration: filter integrity test, bioburden**
Step 12b	**Storage** **Condition: 2°C-8°C**	

[redacted]: Steps [redacted]

a) MVM (minute virus of mice)

b) [redacted]

c) Very low density polyethylene

Process validation of the commercial-scale manufacturing process for the drug substance was carried out and analyses have been performed for the following parameters in addition to the above control items.

Cell growth profile (cell number, viability, population doubling rate, [redacted], [redacted], etc.) and product yield and its quality attributes ([redacted] SDS-PAGE [CBB staining], isoelectric focusing [IEF]) for the fermentation process and the processing time and the percent yield and yield of product for the harvesting step have been determined.

For the purification process, the percent yield of product at each step and monomer content at each chromatographic step and virus removal step have been determined and reducing/non-reducing

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SDS-PAGE and IEF have been carried out at each chromatographic step. Removal of process-related impurities and product-related impurities has been evaluated and host cell protein (HCP), host cell DNA, bovine serum albumin (BSA), antifoaming agents [redacted] and [redacted], and cholesterol are reduced by affinity chromatography, aggregates (dimers), HCP, host cell DNA, BSA, and protein A are reduced by [redacted] ion exchange chromatography (non-adsorbed), and aggregates are reduced by [redacted] ion exchange chromatography (bind and elute) and it has been confirmed that impurities can be controlled at a certain level or lower. Furthermore, re-filtration using a virus removal membrane or a membrane filter for the final sterile filtration has been evaluated at scales of 1/[redacted] or 1/[redacted], respectively, and re-filtration can be carried out up to [redacted] times each, respectively, for each drug substance lot and if re-filtration is actually carried out, its impact on product quality and the integrity of the membrane filter are to be determined. The re-use of each column has also been evaluated and it has been confirmed that at a commercial scale, [redacted] ([redacted] [redacted]) protein A[redacted] column, [redacted] column (non-adsorbed), and [redacted] column (bind and elute) maintain consistent purification performance for up to [redacted], [redacted], and [redacted] re-uses, respectively. The re-use of an ultrafiltration membrane has also been evaluated and it was confirmed that the membrane can be used consistently for up to [redacted] re-uses at a scale of 1/[redacted] of Step 7, which was selected as the worst case scenario, and up to [redacted] re-uses of ultrafiltration membranes for Steps 7 and 11 are allowed. The storage conditions for intermediates obtained at different steps have been established based on the results of stability studies and sterility test, as shown in Table 3.

Table 3. Storage conditions for intermediates obtained at different steps

Intermediate	Container	Maximum allowable holding time	
		[redacted]°C	[redacted]°C
Filtrate after the end of Step 4	Stainless container	[redacted] days	[redacted] hours[a)]
Treated solution after the end of Step 6 Filtrate after the end of Step 7	LDPE bag (after sterile filtration)	[redacted] days	[redacted] days
Non-adsorbed fraction from column after the end of Step 8	Stainless container	[redacted] hours	[redacted] hours
	LDPE bag (after sterile filtration)	[redacted] days	[redacted] days
Filtrate after the end of Step 9	Stainless container	[redacted] hours	[redacted] hours
	LDPE bag (after sterile filtration)	[redacted] days	[redacted] days
Loading solution for column in Step 10	Stainless container	-	[redacted] hours
Column eluate after the end of Step 10	Stainless container LDPE bag (after sterile filtration)	[redacted] hours	[redacted] hours
Drug substance solution after the end of Step 11	Stainless container	[redacted] hours	[redacted] hours

a) Storage temperature is 25°C.

(d) Adventitious agents safety evaluation

i) Non-viral adventitious agents

The raw materials of animal origin used in the drug substance manufacturing process are New Zealand-sourced fetal bovine serum as a medium component in the preparation of the MCB and the preservation of the current WCB and US-sourced BSA and ovine cholesterol sourced from Australia or New Zealand as media components for cell banking and cell culture. These raw materials are derived from healthy animals and the absence of infectious contaminants has been confirmed by the supplier and manufacturer. Moreover, BSA that does not conform to the Standards for Biological Ingredients has been confirmed to meet a threshold to provide safety assurance in accordance with “Handling of Risk

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Assessment etc. in Partial Change Applications for Drugs, Medical Devices, etc., Using Bovine Derived Raw Materials etc." (PFSB/ELD Notification No. 0801001 dated August 1, 2003). A partial change application for switching to BSA sourced from another country will be filed by the 4th quarter of 2011.

ii) Adventitious viruses etc.

Tests of purity were performed on the MCB, WCB, and CAL, and the absence of contamination by mycoplasma, bacteria, infectious retroviruses, and adventitious viruses have been confirmed. Mycoplasma testing, test for the absence of MVM, and *in vitro* assay for adventitious viruses are performed at the unprocessed bulk level in the fermentation process, endotoxin and bioburden are assessed at each purification step, and furthermore, sterility test is performed at the formulation step. Therefore, the manufacturing process of the drug substance is controlled against adventitious agents. The capacity of each purification step to clear viruses is shown in Table 4.

Table 4. Results of viral clearance studies

Purification process	Virus reduction factor (Log_{10})				
	X MuLV	HSV	BAV	POL	MVM
Affinity chromatography	[redacted] a), b)	[redacted] b), c)	[redacted] c), d)	[redacted] c)	[redacted]
Virus inactivation (pH[redacted], [redacted] minutes)	[redacted] c)	[redacted] c)	[redacted]	[redacted]	[redacted]
[redacted]ion exchange chromatography (non-adsorbed)	[redacted] c), d)	[redacted] c), d)	[redacted]	[redacted]	[redacted]
Virus removal (pore size [redacted] nm)	[redacted] c)	[redacted]4 c)	[redacted] c)	[redacted]	[redacted] c)
Overall reduction factor	≥ 16.98 e)	≥ 16.33 e)	≥ 7.66 e)	3.91	4.59

NR: No Reduction, NT: No Tested
BAV (bovine adeno virus), HSV (herpes simplex virus), MVM (minute virus of mice), POL (poliovirus), X MuLV (xenotropic murine leukemia virus)

a) Quantitative PCR
b) As inactivation of eluted viruses by elution buffer (pH[redacted]) has not been confirmed, the reduction factor is not included in an overall reduction factor.
c) 50% tissue culture infectious dose: $TCID_{50}$
d) Virus reduction factor for used resin
e) The sum of the individual factors

(e) Manufacturing process development (Comparability)

The drug substance manufacturing process has undergone four changes during product development (Processes A-D; Process E is the commercial process). Of the main clinical studies in PNH patients, a foreign phase III clinical study (C04-001 [TRIUMPH]), a foreign phase III clinical study (C04-002 [SHEPHERD]), and their extension study (E05-001) used clinical trial material produced by Process D or E and a Japanese clinical study (C07-001) and its extension study (E07-001) used clinical trial material produced by Process E.

The seed cell line for the preparation of the MCB was changed from [redacted] to [redacted] that was of the same origin and had higher expression, which was used in Processes B to E. The fermentation process was scaled up from [redacted] L at the early stage of development to [redacted] L or [redacted] L in Process B, [redacted] L in Processes C and D, and [redacted] L in Process E. In the purification process, protein A derived from *Staphylococcus aureus* to be used in the affinity chromatographic step was changed to [redacted] protein A (genetical recombination) produced without using raw materials of biological origin and pH[redacted] treatment time in the virus inactivation step was changed from [redacted] to [redacted] minutes to [redacted] to [redacted] minutes,

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which were employed in Processes C to E. In order to consistently reduce an increase in aggregates as observed in 1 drug substance lot produced by Process C, [redacted] ion exchange chromatographic step was included in Process D. That step was further changed to [redacted] ion exchange chromatographic step in Process E in order to optimize the process. The pore size of a membrane filter for the virus removal step was changed from [redacted] nm to [redacted] nm, which was employed in Process E.

The comparability of the drug substances before and after manufacturing process changes has been demonstrated by comparing the results of specification tests and characterization studies between post-change drug substance and pre-change reference material.

2.A.(1).2) Structure/Composition

The following characterization studies were performed.

(a) Primary structure

i) Amino acid composition

After hydrolysis, each amino acid was quantified. As a result, the determined amino acid composition agreed with the theoretical values deduced from the cDNA sequence.

ii) N-terminal and C-terminal amino acid sequences

・No heterogeneity of the heavy- and light-chain N-terminal amino acid sequences was detected by Edman degradation. The heavy chain N-terminus was modified with pyroglutamic acid.

・Although no heterogeneity of the light-chain C-terminal amino acid residues was detected by nano-electrospray ionization-tandem mass spectrometry, the C-terminal Gly^{487} or Lys^{488} residue of the heavy chain was detected at a level of [redacted]% or [redacted]%, respectively.

iii) Peptide map

・The protein was reduced, alkylated and digested with trypsin and the obtained peptides were analyzed using reverse-phase chromatography-electrospray ionization-mass spectrometry (RP HPLC-ESI-MS). The results were consistent with the amino acid sequence deduced from cDNA.

・A glycosylation site was identified at Asn298 of the heavy chain CH2 region. No aglycosylation of tryptic peptide T21 (amino acid residues 294-302 of the heavy chain) was detected.

・The deamidation rate was about [redacted]% for T22 (amino acid residues 303-318 of the heavy chain) and [redacted]% for T32 (amino acid residues 372-393 of the heavy chain).

iv) Carbohydrate structure

・Monosaccharide compositional analysis was performed and four monosaccharides (fucose, mannose, galactose [Gal], and *N*-acetylglucosamine [GlcNAc]; about [redacted], [redacted], [redacted], and [redacted] nmol/mg, respectively) were identified and *N*-acetylgalactosamine was not detected, which indicated that eculizumab has no *O*-linked glycosylation. Sialic acid analysis was performed. As a result, the content of

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N-glycolylneuraminic acid (Neu5Gc) was about [redacted] mmol/mol and *N*-acetylneuraminic acid was detected in trace amount, but was below the lower limit of quantification (≤ [redacted] mmol/mol). Carbohydrate constitutes about [redacted]% of the mass of eculizumab.

・Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) or anion exchange chromatography of fluorescently labeled *N*-linked glycans was performed. As a result, fucosylated biantennary *N*-linked glycans with zero, one, or two non-reducing terminal Gal residue(s) (G0F, G1F, G2F; [redacted]%-[redacted]% in total) were mainly detected and glycans lacking the terminal GlcNAc (G0F-GN) and high mannose-type glycans (Man5) were slightly detected. Furthermore, αGal (Galα1-3Gal) glycans were detected at levels of [redacted]% to [redacted]% (size profile of glycans). Neutral and sialylated oligosaccharides were detected at levels of [redacted]% to [redacted]% and [redacted]% to [redacted]%, respectively (glycan charge profile).

(b) Physicochemical properties

i) Molecular weight

MALDI-TOF-MS and ESI-TOF-MS indicated that the molecular weight of the molecule is in agreement with the theoretical molecular mass of 147,870 Da.

ii) Electrophoretic patterns

・IEF showed [redacted] major bands and [redacted] to [redacted] minor bands within a pI range of [redacted] to [redacted].

・SDS-PAGE (CBB staining) yielded two major bands (heavy and light chains) and [redacted] minor bands whose intensities were below the lower limit of quantification (staining intensity, [redacted]%) (antibodies lacking [redacted] light chain, antibodies lacking [redacted] light chain and [redacted] heavy chain [[redacted] form]) under reducing conditions and one major band (monomer, ≥ [redacted]%), [redacted] minor bands (antibodies lacking [redacted] light chain, antibodies lacking [redacted] light chains, ≤ [redacted]% and ≤ [redacted]%, respectively), and [redacted] bands whose intensities were below the lower limit of quantification ([redacted] form, [redacted] different light chain fragments) under non-reducing conditions.

iii) Liquid chromatographic patterns

・Size exclusion chromatography (GE HPLC) yielded a main peak (monomer, ≥ [redacted]%) and a minor high molecular weight peak presumed to represent aggregates (≤ [redacted]%). The molecular weights of monomer and aggregates as determined by LC-ESI-TOF-MS matched the theoretical molecular masses of eculizumab and the dimer, respectively. The potency of aggregates (C5 binding assay) was about [redacted]%. Aggregates produced percent hemolysis of [redacted]% at [redacted] μg/mL and [redacted]% at [redacted] μg/mL, which were lower than monomer ([redacted]% and [redacted]%, respectively).

・Weak acid cation exchange column chromatography yielded one main peak and broad peaks unresolved from the main peak ([redacted] acidic peaks and [redacted] basic peaks).

・Reverse-phase chromatography yielded a single main peak and when the protein was reduced and alkylated, [redacted] main peaks (heavy and light chains) and [redacted] minor peaks were observed.

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・Hydrophobic interaction chromatography yielded a single main peak.

iv) Higher order structure

・The concentration of free sulfhydryl groups in eculizumab as determined by Ellman's assay was below the lower limit of quantification (10 μM).

・Analysis of non-reduced, alkylated tryptic peptides by RP HPLC-ESI-MS confirmed that 28 (14 pairs) out of a total of 36 Cys residues form disulfide bonds within the heavy chain, within the light chain, and between the heavy and light chains (Cys^{22} of the heavy chain-Cys^{96} of the heavy chain, Cys^{136} of the heavy chain-Cys^{214} of the light chain, Cys^{149} of the heavy chain-Cys^{205} of the heavy chain, Cys^{262} of the heavy chain-Cys^{322} of the heavy chain, Cys^{368} of the heavy chain-Cys^{426} of the heavy chain, Cys^{23} of the light chain-Cys^{88} of the light chain, Cys^{134} of the light chain-Cys^{194} of the light chain).

・Near-ultraviolet and far-ultraviolet circular dichroism (CD) yielded the spectra that are characteristic of an antibody. The wavelength of maximum absorption was [redacted] nm and the wavelength of minimum absorption was [redacted] nm. The percentage of β-sheet structure was about [redacted]%.

(c) Biological properties

・The binding affinity of eculizumab for human C5 (dissociation constant), association rate constant, and dissociation rate constant, as determined by surface plasmon resonance, were approximately [redacted] pM, approximately [redacted]$\times 10^{5}$ $M^{-1}s^{-1}$, and approximately [redacted]$\times 10^{-5}$ s^{-1}, respectively, at [redacted]°C and were approximately [redacted] pM, approximately [redacted]$\times 10^{6}$ $M^{-1}s^{-1}$, and approximately [redacted]$\times 10^{-4}$ s^{-1}, respectively, at [redacted]°C.

・The functional complement inhibition activity of eculizumab in human serum was assessed using a hemolytic assay measuring the hemoglobin release from chicken red blood cells. Eculizumab produced percent hemolysis of > [redacted]% at [redacted] μg/mL, but < [redacted]% at [redacted] μg/mL, and almost completely inhibited hemolysis at [redacted] μg/mL.

・Since eculizumab has an IgG_2-IgG_4 heavy chain backbone, eculizumab is not considered to exhibit complement-dependent cytotoxicity activity or antibody-dependent cell-mediated cytotoxicity (ADCC) activity.

・[redacted] mAb epitope mapping ([redacted] and [redacted] using [redacted] or [redacted], and [redacted]] and [redacted] various domains [redacted] and its [redacted]) indicated that [redacted]-terminal [redacted] domain of the human C5α chain is recognized as an epitope.

2.A.(1).3) Product-related substances

Heavy chain N-terminal pyroglutamate, loss of C-terminal lysine residue of the heavy chain, deamidation, oligosaccharide variants, and charge variants have been detected.

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2.A.(1).4) Impurities

(a) Process-related impurities

It has been confirmed that the media components used in the cell culture process, i.e. [redacted], [redacted], and cholesterol are removed to below the lower limit of quantification ([redacted] ppm, [redacted] μg/mL, and [redacted] ng/mL, respectively) by the affinity chromatographic step. Protein A, BSA, HCP, and host cell DNA are reduced in the purification process (< [redacted] ng/mg, < [redacted] ng/mg, < [redacted] ng/mg, and < [redacted] pg/mg, respectively) and listed as impurities in the drug substance specification.

(b) Product-related impurities

Antibody fragments have consistently been detected as minor bands on reducing/non-reducing SDS-PAGE (CBB staining) [see "2.A.(1).2) Structure/Composition (b) Physicochemical properties ii) Electrophoretic patterns"]. The impurities that increase during the shelf-life of the drug substance or the drug product are aggregates identified on reducing/non-reducing SDS-PAGE (CBB staining) and GE HPLC and acidic variants identified by IEF.

2.A.(1).5) Drug substance specification

The proposed specifications for the drug substance are description (appearance), identification (peptide map, size profile of glycans, IEF), osmolality, pH, purity (protein A, BSA, HCP, host cell DNA), polysorbate 80, test for total viable count, bacterial endotoxins, reducing/non-reducing SDS-PAGE (CBB staining), GE HPLC, hemolytic assay, C5 binding assay, and protein concentration.

2.A.(1).6) Stability of drug substance

Using commercial scale lots of the drug substance stored in [redacted] mL LDPE bags, long-term testing (2°C-8°C, 24 months, 3 lots) and accelerated testing ([redacted]°C-[redacted]°C, [redacted] months, 3 lots; [redacted]°C-[redacted]°C, [redacted] months, 1 lot) were conducted and the following stability information was evaluated: description (appearance), peptide map, IEF, osmolarity, pH, reducing/non-reducing SDS-PAGE (CBB staining), GE HPLC, sterility test, bacterial endotoxins, hemolytic assay, C5 binding assay, and protein concentration. At the long-term storage condition, although an increase of aggregates associated with a [redacted]% to [redacted]% decrease of monomers were detected by GE HPLC, there were no marked changes in other attributes tested up to 24 months. At the accelerated condition, the percentage of acidic bands on IEF increased from [redacted]% to [redacted]% at the initial timepoint to [redacted]% to [redacted]%, an increase of aggregates associated with a [redacted]% to [redacted]% decrease of monomers were detected by GE HPLC, and reducing SDS-PAGE (CBB staining) showed about [redacted]% decreases in the heavy and light chains. Since the formulation of the drug product is the same as that of the drug substance, stress testing was performed on the drug product.

Based on the above, a shelf life of 18 months has been proposed for the drug substance when stored in [redacted] L LDPE bags at 2°C to 8°C, protected from light.

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2.A.(2) Drug product

2.A.(2).1) Formulation development

The drug product is an injectable solution and each glass vial contains 300 mg of Eculizumab (Genetical Recombination), the active ingredient, in 30 mL of product solution and the other ingredients are 263.1 mg sodium chloride as an isotonizing agent, 13.8 mg sodium dihydrogenphosphate monohydrate and 53.4 mg disodium hydrogenphosphate heptahydrate as buffering agents, 6.6 mg polysorbate 80 as a stabilizer, and the appropriate amount of water for injection as a solvent. The vial contains an overfill of 1.7 mL.

2.A.(2).2) Drug product manufacturing process

The formulated drug substance is mixed (mixing process), sterile filtered using a hydrophilic membrane filter ([redacted] μm), filled into colorless glass vials, stoppered with siliconized butyl rubber stoppers, and sealed with aluminum seals with polypropylene flip-off caps (sterile filtration/filling/closing process) and packaged (packaging process) and primarily stored at 2°C to 8°C (storage process). The vials are allowed to adjust to room temperature, labelled, and secondary packaged and then stored at 2°C to 8°C (packaging/storage process). The sterile filtration/filling/closing, storage, and packaging/storage processes have been defined as critical process steps. As in-process control tests, test for total viable count during the mixing process, filter integrity test and sterility test during the sterile filtration process, fill weight testing during the filling process, visual inspection during the packaging process, and visual inspection during the packaging/storage process are performed. IEF and hemolytic assay are performed at release. [redacted] to [redacted] at [redacted] ([redacted]) or [redacted] ([redacted]) and [redacted].

During the drug product development, the volume was changed from [redacted] mL to [redacted] mL and [redacted] mL, and then to 30 mL, but the formulation did not change.

2.A.(2).3) Drug product specification

The proposed specifications for the drug product are description (appearance), identification (peptide map), osmolarity, pH, reducing/non-reducing SDS-PAGE (CBB staining), GE HPLC, bacterial endotoxins, sterility test, extractable volume, insoluble particulate matter, foreign insoluble matter, C5 binding assay, and protein concentration.

2.A.(2).4) Stability of drug product

Stability studies of the drug product are summarized in Table 5.

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Table 5. Summary of stability studies of drug product

Stability testing	Test attributes	Test results	
		[redacted]	[redacted]
Long-term testing 2°C-8°C 36 months Protected from light Upright and inverted positions	description (appearance), pH, osmolarity, peptide map, IEF, reducing/non-reducing SDS-PAGE (CBB staining), GE HPLC, sterility test, bacterial endotoxins, insoluble particulate matter, C5 binding assay, protein concentration	3 lots The percentage of acidic bands on IEF increased from [redacted]% at the initial time point to [redacted]% and an increase of aggregates associated with a [redacted]% decrease of monomers were detected by GE HPLC, but there were no marked changes in other attributes tested up to 36 months.	3 lots Stable up to 3 months. Testing will continue up to 36 months.
Accelerated testing 1 20°C-25°C 12 months Protected from light Inverted position	description (appearance), pH, osmolarity, peptide map, IEF, reducing/non-reducing SDS-PAGE (CBB staining), GE HPLC, C5 binding assay, bacterial endotoxins, protein concentration	3 lots As the percentage of the major band on IEF decreased from [redacted]% at the initial time point to [redacted]%, a new band was detected. An increase of aggregates associated with a [redacted]% decrease of monomers were detected by GE HPLC. A new band with a slight increase of aggregates were detected by non-reducing SDS-PAGE. Increases of low and high molecular weight bands with about [redacted]% decreases of heavy and light chains were detected by reducing SDS-PAGE.	—
Accelerated testing 2 23°C-27°C 12 months Protected from light Inverted position	description (appearance), pH, osmolarity, IEF, reducing/non-reducing SDS-PAGE (CBB staining), GE HPLC, hemolytic assay,[1] C5 binding assay, bacterial endotoxins, protein concentration	1 lot Stable up to [redacted] months. Testing will continue up to [redacted] months.	3 lots A slightly broader range of pIs was detected by IEF at [redacted] months, but there were no changes in other attributes tested. Testing will continue up to [redacted] months.
Stress testing[2] (a) Forced degradation low pH, high pH, oxidation, deamidation, storage at high temperature (b) Extreme temperature ・High temperature ([redacted] hours): [redacted]°C-[redacted]°C, [redacted]°C-[redacted]°C ・Low temperature ([redacted] days): [redacted]°C-[redacted]°C (c) [redacted] temperature cycles [redacted]°C-[redacted]°C ([redacted] days) followed by [redacted]°C-[redacted]°C ([redacted] days) [redacted]°C-[redacted]°C ([redacted] days) followed by [redacted]°C-[redacted]°C ([redacted] days) ・[redacted]°C-[redacted]°C ([redacted] days) followed by [redacted]°C-[redacted]°C ([redacted] hours) (d) Photostability Light exposure condition ([redacted] million lx·h, [redacted] W·h/m^2) Ambient temperature (e) Vibration 2°C-8°C	(a)-(e): description (appearance), reducing/non-reducing SDS-PAGE (CBB staining), IEF, GE HPLC (d) (light-protected samples only): pH, osmolarity, insoluble particulate matter (a) and (d) (light-protected samples only) peptide map, hemolytic assay (a), (c)-(e): C5 binding assay (c)-(e): protein concentration	(a) pH[redacted]: Degradation occurred. pH[redacted]: Stable for [redacted] days. Oxidation: Degradation occurred. Deamidation: Degradation occurred. 40°C: Stable for [redacted] hours. (b) Comparable to controls. (c) Comparable to controls. (d) Following light exposure, a decrease of monomers was detected by GE HPLC, a decrease of monomers was detected by non-reducing SDS-PAGE, and a decrease of the major band and a new minor band were detected by IEF. Comparable to controls when protected from light. (e) Comparable to controls.	—

1) Not performed for DSM.
2) Upright position for (b) and inverted position for others.

In addition to the above studies, a preliminary study to explore the storage conditions for reference materials was conducted. As a result, a decrease of monomers was detected by GE HPLC when stored frozen ([redacted]°C, [redacted]°C) vs. refrigerated (2°C-8°C). Therefore, taking account of the impact of prolonged freezing on drug product quality, a shelf-life of 30 months has been proposed for the drug product when stored at 2°C to 8°C, protected from light.

The drug product was diluted with each intravenous solution (Isotonic Sodium Chloride Solution, JP;

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Glucose Injection, JP [5%]; or Ringer's Solution, JP) to the final concentration of 5 mg/mL. The stability of the diluted solutions when stored at 2°C to 8°C or 20°C to 25°C for up to 72 hours was assessed. As a result, there were no marked changes in description (appearance), IEF, reducing/non-reducing SDS-PAGE (CBB staining), GE HPLC, hemolytic assay, or C5 binding assay in each solution.

2.A.(3) Reference materials

The current reference materials, [redacted] and [redacted] are drug substances produced in accordance with the drug substance manufacturing process (Process E) (Lot numbers, [redacted] and [redacted]) and stored at 2°C to 8°C. Reference materials should meet the drug substance specification and should be tested for quality attributes (IEF, reducing/non-reducing SDS-PAGE [CBB staining], GE HPLC, hemolytic assay, C5 binding assay, protein concentration) and characterized (monosaccharide compositional analysis, sialic acid analysis, glycan charge profile, molecular weight [MALDI-TOF-MS, ESI-MS], N-terminal amino acid sequencing, CD).

The tentative shelf-life of the reference materials is [redacted] years and the stability of the reference materials during storage will be assessed by evaluating the quality attributes every [redacted] years and whether the shelf life may be extended will be determined.

A new reference material will be prepared in accordance with the current drug substance manufacturing process based on the shelf-life and the amount left and a newly prepared reference material will be tested for quality attributes and characterized.

2.B Outline of the review by PMDA

2.B.(1) Evaluation of disulfide bond heterogeneity

Though eculizumab has an IgG_2-IgG_4 heavy chain backbone and different patterns of disulfide bonds between Cys residues are expected compared with other IgG subclasses, inter-heavy chain disulfide bonds involving 8 residues in the hinge regions of the two heavy chains (Cys^{224}-Cys^{224}, Cys^{225}-Cys^{225}, Cys^{228}-Cys^{228}, Cys^{231}-Cys^{231}) have not been detected. Thus, PMDA asked the applicant if the patterns of disulfide bonds in eculizumab have been fully analyzed and if their consistency is controlled.

The applicant conducted an additional study, identified peptides containing 9 different hinge regions by ESI-Q-TOF-MS, and presented the structures of 3 different disulfide bond variants deduced from the detected peptides. The applicant responded that although these variants are considered to be consistently present, it is technically difficult to control the variants quantitatively.

Although it is understood that quantitative control is difficult, PMDA is asking the applicant to show their view on the need to control the heterogeneity of these variants, taking account of the possible

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effects of these variants on the safety and efficacy of eculizumab.

2.B.(2) Control of immunogenic glycans

Since eculizumab contains fucosylated biantennary *N*-linked oligosaccharides bearing immunogenic Neu5Gc or αGal (including Galα1-3Gal), PMDA asked the applicant to explain the influences of glycan structure and its heterogeneity on the safety of eculizumab. In terms of ensuring safety, PMDA is also asking the applicant to explain the need to include the Neu5Gc content in the specification and provide a justification for the specification limit for the αGal content.

2.B.(3) C5 binding assay

PMDA asked the applicant to explain the reasons for changing the C5 binding assay method and for variability in assay values despite the principle of the assay being the precise ELISA (enzyme-linked immunosorbent assay) method and provide a justification for the specification limits.

The applicant responded as follows:

Although biotinylated protein L was used for the detection of eculizumab bound to C5 protein in the old assay, as the production of the detection reagents including this reagent and the detector was discontinued, a new assay has been employed, which uses HRP (horseradish peroxidase)-labeled mouse anti-human IgG_4 antibody for detection. Although the values obtained with the new assay are about [redacted]% lower than those obtained with the old assay, the robustness of the new assay has been demonstrated through a validation study. The values obtained with the new assay for the internal standard, the clinical formulation, and the proposed commercial formulation were within a range of 800,000 to 1,200,000 BU/mg, as with the old assay. The change to the new assay was approved by EMEA in April 2009 and FDA in June 2009.

PMDA is asking the applicant to provide a justification for the specification limits, taking account of the values obtained with the new assay for the clinical formulation and the proposed commercial formulation that have been used in patients.

2.B.(4) Drug product specification

2.B.(4).1) Inclusion of foreign insoluble matter test

PMDA asked the applicant to consider including Foreign Insoluble Matter Test for Injections, JP in the drug product specification.

The applicant responded that although foreign insoluble matters have never been detected so far, Foreign Insoluble Matter Test will be included in the specification.

PMDA accepted the response.

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2.A.(4).2) Inclusion of IEF and hemolytic assay

PMDA is asking the applicant to explain the reason for not including IEF and hemolytic assay in the specification in Japan despite IEF and hemolytic assay being included in the drug product specification in the US and Europe and the basis for considering that the quality of the drug product can be assured by the results of these tests performed at the time of its release.

3. Non-clinical data

3.(i) Summary of pharmacology studies

3.(i).A Summary of the submitted data

3.(i).A.(1) Primary pharmacodynamics

There are three pathways of complement activation and C3a and C3b are formed from C3 in all three pathways. C3b cleaves C5 into C5a and C5b. C5a has anaphylatoxin activity and leukocyte chemotactic activity and induces mast cell degranulation, vascular hyperpermeability, and smooth muscle contraction etc. C5b interacts with C6 to C9, allowing for the formation of the complex C5b-9 and C5b-9 causes cell lysis. As eculizumab is an anti-C5 antibody, the potency of each anti-C5 antibody was assessed by measuring hemolytic activity* and C5a generation.

3.(i).A.(1).1) Generation of eculizumab (4.2.1.1.1-4.2.1.1.3, Studies GTR49, GTR56, and GTR52)

Murine anti-human C5 (hC5) mAbs were screened and m5G1.1 mAb as an inhibitor of both hemolytic activity (complete inhibition at [redacted] μg/mL) and C5a generation (complete inhibition at [redacted] μg/mL) was selected. The K_D of m5G1.1 mAb for hC5 was 29.5 pmol/L.

In order to reduce immunogenicity in humans, the CDRs of m5G1.1 mAb were inserted into a human antibody (h5G1.1) and using the Fab region of the generated antibody (h5G1.1 Fab (CDR)), the capacity to compete with m5G1.1 mAb for binding to hC5 was compared. As a result, the Fab region of m5G1.1 mAb and h5G1.1 Fab (CDR) exhibited similar capacities to compete with m5G1.1 mAb for binding to hC5 at [redacted] ng/mL to [redacted] μg/mL. Thus, the applicant explained that humanization of the framework regions had no effect on the hC5 binding capacity of the antibody.

For further humanization, an antibody containing the variable regions of h5G1.1 Fab (CDR), human κ light-chain constant regions, and hybrid human IgG_2-IgG_4 heavy-chain constant regions, which have been reported to be unable to activate complement or bind to the Fc-γ receptor of effector cells (h5G1.1 G2/G4 mAb: eculizumab), was generated.

* Chicken erythrocytes sensitized with anti-chicken erythrocyte antibody were added to human serum for hemolysis.

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3.(i).A.(1).2) C5 inhibition activity of eculizumab (4.2.1.1.4 and 4.2.1.1.6, Studies GTR84 and BP 26FR)

Eculizumab, h5G1.1 G4 mAb[†], and m5G1.1 mAb inhibited human serum-mediated hemolysis of chicken erythrocytes sensitized with anti-chicken erythrocyte antibody (almost complete inhibition at 100 μg/mL, [redacted] μg/mL, and [redacted] μg/mL, respectively) and the applicant explained that the difference in the heavy-chain constant regions were shown not to affect C5 inhibition activity.

In another study, eculizumab almost completely blocked human serum-mediated hemolysis of chicken erythrocytes sensitized with anti-chicken erythrocyte antibody at 25 μg/mL, but did not inhibit hemolysis in rhesus monkey, baboon, chimpanzee, cynomolgus monkey, pig, rabbit, rat, or guinea pig sera even at 100 μg/mL.

3.(i).A.(1).3) Affinity of eculizumab for hC5 (4.2.1.1.5, Study GTR109)

The K_D of eculizumab for hC5 (mean ± standard deviation [SD]) was 46 ± 1.6 pmol/L (25°C) or 120 ± 5.5 pmol/L (37°C).

3.(i).A.(1).4) Epitope mapping of hC5 (4.2.1.1.7, Study GTR55)

Epitope mapping of hC5 using m5G1.1 mAb was performed, which indicated that the antibody recognizes the amino acid sequences at positions 822 to 826 (DVFLE), 879 to 883 (KSSKC), and 930 to 933 (VPEG) as an epitope cluster.

3.(i).A.(2) Secondary pharmacodynamics

3.(i).A.(2).1) Cross-reactivity with human tissues (4.2.1.2.1, Study PAI-IM1184)

The potential cross-reactivity of eculizumab (5 and 30 μg/mL) with cryosections of normal human tissues was evaluated by immunostaining. As a result, eculizumab staining was observed in smooth and striated muscle in various tissues, myoepithelium, myofibroblasts, renal tubular epithelium, reticulum cells, and platelets etc. The applicant discussed that eculizumab is not considered to have cross-reacted with human tissues because (a) C5 expression in vascular smooth muscle cells and heart muscle have been demonstrated (*Microbiol Immunol.* 1999;43:585-593, *Circ Res.* 1998;83:860-869.), (b) C5 is a secreted protein and is not expressed on cell surface (*Annu Rev Immunol.* 1995;13:277-305.), and (c) the staining likely represents staining of intracellular C5 as acetone fixation and cryotomy of the tissue/cell samples expose intracellular sites.

3.(i).A.(3) Safety pharmacology

Since eculizumab has high selectivity for hC5 and is species-specific, no safety pharmacology studies have been conducted.

[†] An antibody containing the variable regions of h5G1.1 Fab (CDR), human κ light-chain constant regions and human IgG4 heavy-chain constant regions

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3.(i).B Outline of the review by PMDA

3.(i).B.(1) Efficacy of eculizumab in PNH

PNH arises from the deficiency of the GPI-anchored terminal complement inhibitor CD59 on hematopoietic stem cells. Since CD59 inhibits C9 binding to complement C5 and blocks the formation of the terminal complement complex C5b-9 on the RBC surface, the deficiency of CD59 on PNH RBCs leads to deposition of cytolytic C5b-9 on the surface, which results in hemolysis.

Eculizumab binds to hC5, preventing the formation of C5b-9 by inhibiting the cleavage of C5 and is expected to reduce C5b-9-mediated hemolysis.

Although non-clinical pharmacology studies submitted in the application have not demonstrated hemolytic inhibitory activity *in vivo*, as there is no animal model of PNH and eculizumab was created as an antibody against hC5 and did not inhibit hemolysis in non-human sera, suggesting that eculizumab does not inhibit C5 in sera of other species, the lack of a non-clinical *in vivo* study to demonstrate efficacy of eculizumab was unavoidable. On the other hand, since *in vitro* studies using eculizumab showed that eculizumab binds to hC5 and inhibits human serum-mediated hemolysis, it can be inferred that eculizumab reduces hemolysis by inhibiting the cleavage of C5 and preventing the formation of C5b-9.

However, many of *in vitro* studies were performed once. Data from repeated studies were needed for explanation in order to check the reproducibility of studies.

3.(i).B.(2) Cross-reactivity of eculizumab

PMDA's view on the applicant's discussion that eculizumab does not cross-react with various human tissues is as follows:

It has been reported that C5 is a secreted protein and is not expressed on cell surface and it has also been explained that secreted proteins were fully removed. Thus, an inference that the observed staining was due to the binding of eculizumab to intracellular C5 is understandable. However, since the cross-reactivity study of eculizumab with human tissues was conducted by the methods that expose intracellular sites, it is difficult to determine whether eculizumab staining of tissues represents eculizumab binding to cell surface or intracellular sites of tissues. If eculizumab cross-reacts with the cell surface of tissues, there should be potentially some effects on biological functions. However, except for headache, there were no particular adverse events reported at a higher incidence by the eculizumab group than by the placebo group in a clinical study (C04-001 [TRIUMPH]). The applicant discussed the mechanism of eculizumab-induced headache as follows: hemolysis inhibition by eculizumab leads to reduced free Hb, which scavenges nitric oxide (NO), and increased free NO dilates blood vessels, causing headache. However, as the mechanism is undefined, the possible involvement of the cross-reactivity of eculizumab can not be excluded.

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PMDA asked the applicant about findings on the cross-reactivity of eculizumab with complements other than C5 or cytokines. The applicant responded that no cross-reactivity study of eculizumab with complements other than C5 or cytokines had been conducted.

PMDA considers as follows:
If eculizumab cross-reacts with complements other than C5 or cytokines, the immune function will be affected. However, as no marked increase in the incidence of infection-related adverse events was observed in the clinical study (C04-001 [TRIUMPH]), the possibility that the cross-reactivity of eculizumab with complements other than C5 or cytokines leads to clinically immediately relevant events has not been suggested so far.

3.(i).B.(3) Safety pharmacology
Since no safety pharmacology studies of eculizumab have been performed, PMDA asked the applicant to explain the effects of eculizumab on the central nervous, cardiovascular, and respiratory systems based on the results from toxicity and clinical studies, etc.

The applicant explained as follows:
In a 26-week intravenous injection toxicity study in mice of a murine anti-C5 antibody, BB5.1 mAb, there were no effects on the central nervous, cardiovascular, or respiratory systems at the doses inhibiting hemolytic activity [see "3.(iii).A.(2) Repeat-dose toxicity"]. In clinical studies, except for headache, there were few adverse events involving central nervous, cardiovascular, or respiratory system, etc. Therefore, it is considered that eculizumab has no significant effects on the central nervous, cardiovascular, or respiratory systems.

PMDA considers as follows:
Since eculizumab was developed as an antibody against hC5 and there is no cross-reactivity of eculizumab with C5 from other species and eculizumab is a humanized antibody except for the CDRs, it is understood that safety pharmacology evaluation is difficult. On the other hand, there were no treatment-related effects on the central nervous, cardiovascular, or respiratory systems at the doses inhibiting hemolytic activity in the 26-week repeat-dose toxicity study in mice of a murine anti-C5 antibody, BB5.1 mAb. Thus, non-clinical data indicate that there are no effects of C5 inhibition on the central nervous, cardiovascular, and respiratory systems.

See "4.(iii).B.(5) Safety" for the safety of eculizumab in clinical studies.

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3.(ii) Summary of pharmacokinetic studies

3.(ii).A Summary of the submitted data

Since eculizumab binds specifically to hC5, a C5-deficient mouse model reconstituted with hC5, and an IgG_4 isotype of eculizumab (h5G1.1 G4 mAb), which is identical in amino acid sequence to eculizumab except that it contains the human IgG_4 heavy-chain constant region, instead of eculizumab, were used to determine the serum concentrations of the antibody over time and assess hemolytic activity.[‡]

The hC5 and h5G1.1 G4 mAb levels were determined by ELISA assay.

3.(ii).A.(1) Absorption

3.(ii).A.(1).1) Single-dose studies (4.2.2.2.1, Studies GTR-0104.00 and GTR-0104.01)

Male mice were intravenously or subcutaneously injected a single dose of h5G1.1 G4 mAb at 5, 17, 50, 100, or 150 µg, or 190 µg of 2A2 HuG4[§] as a negative control antibody, or vehicle (0.2 mL phosphate-buffered saline) and hemolytic activity inhibition was assessed for up to 48 hours after injection. Hemolytic activity inhibition was detected from 2 minutes until 48 hours following the intravenous administration of 50 to 150 µg of h5G1.1 G4 mAb and hemolytic activity inhibition was detected, but delayed by 24 hours following the subcutaneous injection of 50 µg.

Female mice were intravenously or subcutaneously injected a single dose of 50 µg of h5G1.1 G4 mAb or 60 µg of 2A2 HuG4 and serum h5G1.1 G4 mAb and 2A2 HuG4 concentrations over time were determined. Serum h5G1.1 G4 mAb concentrations over time following the single intravenous or subcutaneous administration of 50 µg of h5G1.1 G4 mAb are shown in Figure 1.

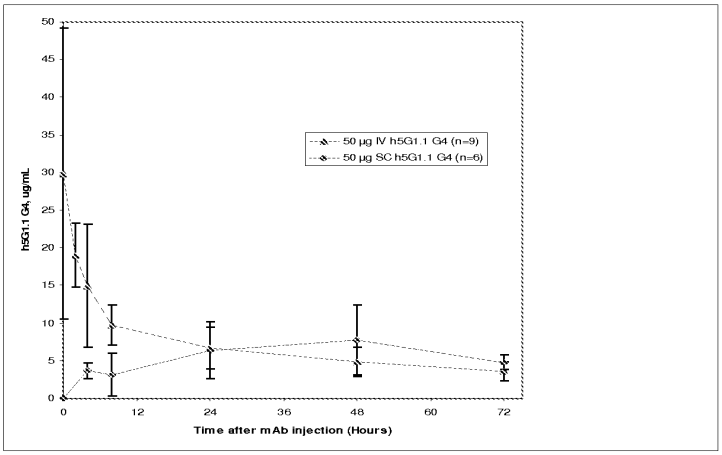

Figure 1. Serum h5G1.1 G4 mAb concentrations over time following single administration of h5G1.1 G4 mAb

‡ Chicken erythrocytes sensitized with anti-chicken erythrocyte antibody were added to mouse serum for hemolysis.

§ An unrelated antibody with a human IgG_4 heavy-chain constant region

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3.(ii).A.(2) Distribution, metabolism, excretion, and pharmacokinetic drug interactions

As eculizumab and h5G1.1 G4 mAb bind to a soluble protein, hC5, which is secreted into the blood and do not bind to murine C5, a distribution (organ/tissue distribution, protein binding, placental transfer, etc.), metabolism, excretion, or pharmacokinetic drug interaction study has not been performed.

The applicant discussed as follows:

Generally, placental transfer of maternal IgG antibodies to the fetus is known and antibodies are excreted in milk. Thus, eculizumab is also expected to cross the placenta and be excreted in milk.

3.(ii).B Outline of the review by PMDA

3.(ii).B.(1) Non-clinical pharmacokinetic studies

PMDA considers as follows:

Since eculizumab, developed as an antibody against hC5, does not demonstrate cross-reactivity with C5 from other species and is a humanized antibody except for the CDRs, the lack of non-clinical pharmacokinetic assessment of eculizumab was unavoidable. The h5G1.1 G4 mAb concentrations over time determined in mice are just reference information because a C5-deficient mouse model reconstituted with hC5 was used and the heavy-chain constant region structure of h5G1.1 G4 mAb, which was used instead of eculizumab, is different from that of eculizumab, and it is difficult to predict the pharmacokinetics of eculizumab in humans.

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

Since eculizumab binds specifically to hC5 and did not inhibit hemolysis in sera from the non-human animals studied [see "3.(i).A.(1).2) C5 inhibition activity of eculizumab"], a non-clinical toxicity study of eculizumab has not been performed. However, using a surrogate murine anti-murine C5 monoclonal antibody, BB5.1 mAb, 26-week repeat-dose and reproductive and developmental toxicity studies in mice were conducted to evaluate the long-term treatment effects of C5 inhibition on tissue toxicity and reproductive function. BB5.1 mAb is a full-length monoclonal antibody of IgG_1 isotype and its amino acid sequence is different from that of eculizumab, but publications (*J Clin Invest*. 2005;115:1590-1600, etc.) have reported that BB5.1 mAb binds specifically to murine C5 and blocks C5b-9 activity and C5a activity *in vitro* and *in vivo*.

For serum BB5.1 mAb concentrations, percent hemolysis at each timepoint was measured using a serum complement hemolytic activity assay system [see "3.(i).A.(1).2) C5 inhibition activity of eculizumab"] and a lower percent hemolysis compared with the control group was a qualitative indicator of systemic exposure.

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3.(iii).A.(1) Single-dose toxicity

No single-dose studies have been conducted.

3.(iii).A.(2) Repeat-dose toxicity (4.2.3.2-1 and 4.2.3.2-2, Studies 6709-108 and 6709-109)

Female mice were intravenously administered vehicle (20 mM Tris-buffered saline three times weekly) or 30 mg/kg of BB5.1 mAb once, twice, or three times weekly (30, 60, or 90 mg/kg/week) for 4 weeks. No toxic signs were observed. The percent hemolysis was 92% to 94% in the control group, 3% to 34% in the once weekly (30 mg/kg/week) group, 1% to 13% in the twice weekly (60 mg/kg/week) group, and 0% to 15% in the three times weekly (90 mg/kg/week) group. Since hemolytic activity was similar for mice treated with BB5.1 mAb twice weekly (60 mg/kg/week) and three times weekly (90 mg/kg/week), it was concluded that the highest dose of BB5.1 mAb in future toxicology studies is 60 mg/kg/week.

Female or male mice were intravenously administered vehicle (20 mM Tris-buffered saline twice weekly) or 30 mg/kg of BB5.1 mAb once or twice weekly (30 or 60 mg/kg/week) for 26 weeks (a 4-week recovery period was scheduled for mice in the control and 60 mg/kg/week groups). Five mice (3 males and 2 females) in the 60 mg/kg/week group died and the cause of deaths was unknown. Even in the control group, 4 deaths (3 males and 1 female) occurred. As there were no toxicologically significant changes, the no observed adverse effect level (NOAEL) was determined to be 60 mg/kg/week.

The mean percent hemolysis was 68.3% to 86.6% in the control group (including prior to initiation of treatment and after a 4-week recovery), 13.3% to 18.8% in the 30 mg/kg/week group (during treatment), and 3.9% to 21.0% in the 60 mg/kg/week group (during treatment). The mean percent hemolysis was 41.7% in females and 67.5% in males (after a 4-week recovery) and there was no particular gender-related difference.

3.(iii).A.(3) Genotoxicity and carcinogenicity

Eculizumab is specific to hC5 and does not have cell proliferative activity or growth factor-like activity and it was considered that genotoxic substances are unlikely to arise from the manufacturing process of eculizumab. Thus, no genotoxicity or carcinogenicity studies have been conducted.

3.(iii).A.(4) Reproductive and developmental toxicity

Although the placental transfer of eculizumab or BB5.1 mAb has not been studied, as BB5.1 mAb is an IgG_1 antibody, it is inferred that it may cross the placenta.

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3.(iii).A.(4).1) Study of fertility and early embryonic development to implantation (4.2.3.5.1.1, Study 6709-104)

Mice were intravenously administered vehicle (20 mM Tris-buffered saline twice weekly) or 30 mg/kg of BB5.1 mAb once or twice weekly (30 or 60 mg/kg/week). Male mice were dosed from 4 weeks prior to mating until 1 or 2 days prior to necropsy and female mice were dosed from 2 weeks prior to mating, throughout the mating period, and until gestation day 7. Although dose-related, reduced body weight gain, reduced food consumption, and decreased prostate gland weight were observed in males, there were no abnormalities in copulation index, sperm motility, or epididymal sperm count, etc. There were no abnormalities in clinical signs or reproductive performance in females. The NOAELs for parental general toxicity, parental reproductive toxicity, and embryo-fetal viability were all determined to be 60 mg/kg/week.

The percent hemolysis (mean ± SD) was 90.2 ± 11.9% (at Week 4) and 100.0 ± 0.0% (at Week 10) in males and 98.4 ± 3.0% (on gestation day 12) in females in the control group, 24.6 ± 16.5% (at Week 4) and 27.2 ± 14.6% (at Week 10) in males and 50.6 ± 46.0% (on gestation day 12) in females in the 30 mg/kg/week group, and 7.0 ± 6.2% (at Week 4) and 16.2 ± 10.1% (at Week 10) in males and 50.6 ± 29.5% (on gestation day 12) in females in the 60 mg/kg/week group.

3.(iii).A.(4).2) Embryofetal development study (4.2.3.5.2.1, Study 6709-105)

Pregnant mice were intravenously administered vehicle (20 mM Tris-buffered saline twice weekly) or 30 mg/kg of BB5.1 mAb once or twice weekly (30 or 60 mg/kg/week) from gestation day 6 to gestation day 15. There were no abnormal findings in dams. In fetuses, one case of malrotated hindlimbs and one case of cleft palate were observed at 30 mg/kg/week, but not at 60 mg/kg/week and therefore, these findings were considered unrelated to BB5.1 mAb. At 60 mg/kg/week, one case of umbilical hernia and two cases (from two different litters) of unilateral retinal dysplasia (folded retina: a severe fold or multiple folds of the retina) were observed and these findings were considered related to BB5.1 mAb.

Evaluations for the umbilical hernia and folded retinas observed in mouse fetuses were performed by an external consultant. According to a report by Research Triangle Institute international, a laboratory and research organization (RTI project No. 08412.007), these findings did not occur. There is a case in which fetal retinal folds were shown to be artifacts produced by the process of tissue preparation (*Toxicol Appl Pharmacol.* 1976;35:347-354.) and although congenital retinal dysplasia should be usually bilateral, the retinal dysplasia was unilateral in this study and there were no other intraocular abnormalities or haemorrhage, etc. Therefore, the applicant explained that the folded retinas may have been artifacts. The development of umbilical hernia was assessed as unknown relationship to BB5.1 mAb.

The NOAEL was determined to be 60 mg/kg/week for maternal general and reproductive toxicity and

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the NOAEL for fetal developmental toxicity was determined to be 30 mg/kg/week as umbilical hernia was noted at 60 mg/kg/week.

The percent hemolysis (mean ± SD) (on gestation day 18) was 78.0 ± 43.8% in the control group, 52.6 ± 49.0% in the 30 mg/kg/week group, and 62.4 ± 37.8% in the 60 mg/kg/week group.

3.(iii).A.(4).3) Study for effects on pre- and postnatal development, including maternal function (4.2.3.5.3.1, Study 6709-107)

Pregnant mice were intravenously administered vehicle (20 mM Tris-buffered saline twice weekly) or 30 mg/kg of BB5.1 mAb once or twice weekly (30 or 60 mg/kg/week) from gestation day 6 to lactation day 18. There were no abnormal findings in maternal animals or pups and the NOAELs for maternal general toxicity and reproductive performance and for F_1 pups were both determined to be 60 mg/kg/week.

The percent hemolysis (mean ± SD) (on lactation day 14) was 95.6 ± 7.0% in the control group, 80.6 ± 20.5% in the 30 mg/kg/week group, and 83.6 ± 16.6% in the 60 mg/kg/week group.

Pregnant and lactating mice in the above 1) to 3) reproductive and developmental toxicity studies exhibited higher percent hemolysis (50.6%-80.6% at 30 mg/kg/week and 50.6%-83.6% at 60 mg/kg/week [mean]) than the non-pregnant female mice in the 26-week repeat-dose toxicity study (13.3%-14.5% at 30 mg/kg/week and 3.9%-4.6% at 60 mg/kg/week). The applicant inferred that pregnancy and lactation may have affected the amount of complement or the complement inhibitory function of the antibody, but the cause is unknown.

3.(iii).A.(5) Local tolerance

No local tolerance studies have been performed with eculizumab or other anti-C5 antibodies. However, BB5.1 mAb or vehicle-related local irritation at the injection site was not observed in repeat-dose or reproductive and developmental toxicity studies.

3.(iii).B Outline of the review by PMDA

3.(iii).B.(1) Retinal dysplasia

PMDA's view on the retinal dysplasia observed in an embryofetal development study is as follows:

The conducting laboratory concluded that the folded retinas were due to a teratogenic effect of BB5.1 mAb, whereas the applicant explained that the folded retinas were not related to BB5.1 mAb, based on the inference mainly from the background data for the cases of folded retina and the case of artifacts. However, histopathological findings of the retinas, hemolytic inhibition in the dams of normal fetuses vs. the dams of the fetuses with folded retinas, or receptor distribution in ocular tissue, etc. have not been examined based on the data from this study. Therefore, the applicant's explanation that the folded

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retinas were not related to BB5.1 mAb is not based on solid evidence and like umbilical hernia, whether the folded retinas were related to BB5.1 mAb or not is unknown.

Umbilical hernia and folded retinas were observed in the high dose group (60 mg/kg/week) only and the incidence of umbilical hernia was 0.4% (1 of 230 offspring) and the incidence of folded retina was 1.7% (2 of 114 offspring). In addition, the two cases of folded retina were unilateral and there were no abnormalities in clinical observations or necropsy findings of offspring born to mothers exposed to BB5.1 mAb (388 offspring) in a study for effects on pre- and postnatal development, including maternal function (6709-107). Furthermore, no effects on the eyes of the offspring of women who were treated with eculizumab during pregnancy have been reported in clinical studies or foreign post-marketing surveillance [see "4.(iii).B.(8).2) Use during pregnancy or lactation"]. Taking account of the above, although it is necessary to provide information on these findings, there is little need for further investigation.

3.(iii).B.(2) Toxicity studies using an anti-C5 antibody other than eculizumab

PMDA's view on the use of an anti-C5 antibody other than eculizumab in toxicity studies submitted in the application is as follows:

Although the amino acid sequence of either the variable or constant region of BB5.1 mAb, an anti-C5 antibody used in place of eculizumab, is not similar to that of eculizumab, it has been reported that BB5.1 mAb binds specifically to murine C5 and inhibits hemolytic activity in mice (*J Clin Invest*. 2005; 115:1590-1600, etc.). Toxicity studies with BB5.1 mAb evaluated chronic effects and reproductive and developmental effects at doses that demonstrated the reduction of the mean percent hemolysis by C5 inhibition, which showed no toxicity findings. Therefore, C5 inhibition is unlikely to result in chronic toxicity or reproductive and developmental toxicity. As no toxicity studies with eculizumab have been conducted, the effects of eculizumab other than those of C5 inhibition are unknown.

However, since eculizumab does not cross-react with C5 from other species, toxicological evaluation based on studies with eculizumab is difficult. Therefore, toxicological evaluation is inevitably based on studies with BB5.1 mAb.

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4. Clinical data

4.(i) Summary of biopharmaceutic studies and associated analytical methods

The to-be-marketed formulation was used in all clinical studies submitted as evaluation data in the application.

Concentrations of eculizumab, soluble C5b-9 (sC5b-9) and human anti-human antibodies (HAHA) in human serum were measured using an ELISA method and an electro-chemiluminescent immunoassay.

No data from biopharmaceutic studies have been submitted.

4.(ii) Summary of clinical pharmacology studies

4.(ii).A Summary of the submitted data

4.(ii).A.(1) Studies using human biomaterials

No data from studies using human biomaterials have been submitted.

4.(ii).A.(2) Japanese phase II clinical study (5.3.4.2.6 and 5.3.5.2.6, Study C07-001 [20 to 20])

See "4.(iii).A.(1) Japanese phase II clinical study (C07-001)" for a brief description of the study.

Pharmacokinetic analysis showed that the trough serum concentrations of eculizumab (mean ± standard error [SE]) at Week 2 and Week 12 were 90.5 ± 6.25 μg/mL and 116.5 ± 10.93 μg/mL, respectively. The pharmacokinetic parameters estimated by a population pharmacokinetic (PPK) analysis with a 1-compartmental model are shown in Table 6.

Table 6. Estimated pharmacokinetic parameters using a 1-compartmental model

CL (L/h)	V_d (L)	K_{el} (1/h)	$t_{1/2}$ (h)
0.0173 ± 0.00633	6.09 ± 1.417	0.00288 ± 0.000928	265.6 ± 83.9

Mean ± SD, n = 26

Pharmacodynamic analysis showed that the proportion of patients with hemolytic activity values ≤ 20%** was 62.1% (18 of 29 patients) at Week 1 and reached 93.1% (27 of 29 patients) at Week 8 and at Week 12. The pharmacodynamic parameters for hemolytic activity estimated using the cumulative Weibull function are shown in Table 7.

Table 7. Estimated pharmacodynamic parameters using the cumulative Weibull function

E_0 (%)	E_{diff} (%)	C_{deff} (μg/mL)	sf
100.2 ± 34.54	96.7 ± 33.34	36.5 ± 18.05	2.61

Mean ± SD, n = 27

E_0: hemolytic activity at baseline, E_{diff}: theoretical maximal inhibitory effect,
C_{deff}: eculizumab concentration required to produce 1/e (36.8%) effect, sf: slope of PK/PD relationship

** At least 100 serum samples from healthy adults were assayed for hemolytic activity and a cutoff value of 20% ("mean minus 3SD") was chosen based on the idea that hemolytic activity of ≤ 20% represents complete blockade of terminal complement activity in serum.

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4.(ii).A.(3) Foreign phase I clinical study (5.3.5.2.1, Study C02-001 [May 2002 to January 2003]; *N Engl J Med.* 2004;350:552-558.)

See "4.(iii).A.(3) Foreign phase I clinical study (C02-001)" for a brief description of the study.

Pharmacokinetic analysis showed that the trough serum concentrations of eculizumab (mean ± SD) at Week 10 and Week 12 were 108.1 ± 55.33 μg/mL and 70.2 ± 20.13 μg/mL, respectively, and the peak serum concentrations of eculizumab at Week 4 and Week 10 were 259.12 ± 51.47 μg/mL and 201.09 ± 96.28 μg/mL, respectively, and the applicant explained that serum concentrations did not reach a steady-state by Week 12.

Pharmacodynamic analysis showed that ≤ 20% hemolytic activity was maintained from Week 3 to Week 12 in 90.9% of patients (10 of 11 patients).

4.(ii).A.(4) Foreign phase I clinical study (5.3.5.2.2, Study E02-001 [August 2002 to December 2003]; *Blood*. 2005;106:2559-2565.)

See "4.(iii).A.(4) Foreign phase I clinical study (E02-001)" for a brief description of the study.

Pharmacokinetic analysis showed that the trough serum concentrations of eculizumab (mean ± SD) at baseline (after 12 weeks of treatment), Week 28 (after 40 weeks of treatment), and Week 52 (after 64 weeks of treatment) were 81.8 ± 48.54 μg/mL, 166.9 ± 102.1 μg/mL, and 112.4 ± 46.88 μg/mL, respectively.

Pharmacodynamic analysis showed that the level of hemolytic activity was ≤ 20% at all timepoints in all patients up to Week 52.

4.(ii).A.(5) Foreign phase II clinical studies (5.3.5.2.3, Studies X03-001 and X03-001A [August 2003 to December 2005]; *Haematologica*. 2005;90:ECR40.)

See "4.(iii).A.(5) Foreign phase II clinical studies (X03-001 and X03-001A)" for a brief description of the studies.

Pharmacokinetic analysis showed that the trough serum concentrations of eculizumab (mean ± SD) at baseline (after 64 weeks of treatment) and Week 50 (after 114 weeks of treatment) were 106.8 ± 47.62 μg/mL and 80.5 ± 37.26 μg/mL, respectively.

Pharmacodynamic analysis showed that although the level of hemolytic activity exceeded 20% in one patient each at Weeks 4, 42, and 50, the level of hemolytic activity was ≤ 20% at all timepoints in the remaining patients.

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4.(ii).A.(6) Foreign phase III clinical study (5.3.5.1.1, Study C04-001 [TRIUMPH] [August 2004 to December 2005]; *Br J Haematol.* 2008;142:263-272, *N Engl J Med.* 2006;355:1233-1243.)

See "4.(iii).A.(6) Foreign phase III clinical study (C04-001)" for a brief description of the study.

Pharmacokinetic analysis showed that the trough serum concentrations of eculizumab (mean ± SE) in the eculizumab and placebo groups were 113.5 ± 8.70 μg/mL and 10.1 ± 0.08 μg/mL, respectively, at Week 4 and 101.8 ± 10.8 μg/mL and 10.1 ± 0.09 μg/mL, respectively, at Week 26.

Pharmacodynamic analysis showed that the proportions of patients with hemolytic activity values ≤ 20% at Week 4 and Week 26 were 100.0% (42 of 42 patients) and 97.6% (41 of 42 patients), respectively.

4.(ii).A.(7) Foreign phase III clinical study (5.3.4.2.5 and 5.3.5.2.4, Study C04-002 [SHEPHERD] [December 2004 to October 2006]; *Blood.* 2008;111: 1840-1847, *Br J Haematol.* 2008;142:263-272.)

See "4.(iii).A.(7) Foreign phase III clinical study (C04-002)" for a brief description of the study.

Pharmacokinetic analysis showed that the trough serum concentrations of eculizumab (mean ± SE) at Week 4 and Week 52 were 104.5 ± 5.08 μg/mL and 110.3 ± 8.92 μg/mL, respectively. The pharmacokinetic parameters estimated by a PPK analysis with a 1-compartmental model are shown in Table 8.

Table 8. Estimated pharmacokinetic parameters using a 1-compartmental model

CL (mL/h/kg)	V_d (mL/kg)	K_{el} (1/h)	$t_{1/2}$ (h)
0.3349 ± 0.13401	113.93 ± 28.719	0.003077 ± 0.0013682	261.07 ± 90.795

Mean ± SD, n = 97

Pharmacodynamic analysis showed that the proportions of patients with hemolytic activity values ≤ 20% at Week 4 and Week 52 were 95.8% (91 of 95 patients) and 84.9% (79 of 93 patients), respectively. The pharmacodynamic parameters for hemolytic activity estimated using an E_{max} model with Hill coefficient are shown in Table 9. The applicant discussed that the very large Hill coefficient indicated that eculizumab inhibits C5 at low concentrations.

Table 9. Estimated pharmacodynamic parameters using an E_{max} model with Hill coefficient

E_0 (%)	E_{max} (%)	EC_{50} (μg/mL)	Hill coefficient
90.14 ± 4.418	88.14 ± 4.583	31.509 ± 7.8152	7.2521 ± 2.82334

Mean ± SD, n = 97
E_0: hemolytic activity at baseline, E_{max}: theoretical maximal inhibitory effect
EC_{50}: eculizumab concentration required to produce 50% of E_{max}

4.(ii).A.(8) Foreign phase III clinical study (5.3.4.2.4 and 5.3.5.2.5, Study E05-001 [May 2005 to March 2006 (Interim report)])

See "4.(iii).A.(8) Foreign phase III clinical study (E05-001)" for a brief description of the study.

The pharmacokinetic parameters estimated by a PPK analysis with a 1-compartmental model are shown

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in Table 10.

Table 10. Estimated pharmacokinetic parameters using a 1-compartmental model

CL (mL/h/kg)	V_d (mL/kg)	K_{el} (1/h)	$t_{1/2}$ (h)
0.329 (39.0%)	113.0 (23.7%)	0.0030 (41.4%)	263 (34.2%)

Mean (CV%), n = 141

The pharmacodynamic parameters for hemolytic activity estimated using the cumulative Weibull function are shown in Table 11.

Table 11. Estimated pharmacodynamic parameters using the cumulative Weibull function

E_0 (%)	E_{diff} (%)	C_{deff} (μg/mL)	sf
93.8 (9.35%)	91.0 (9.56%)	30.6 (27.2%)	2.61 (43.5%)

Mean (CV%), n = 141
E_0: hemolytic activity at baseline, E_{diff}: theoretical maximal inhibitory effect
C_{deff}: eculizumab concentration required to produce 1/e (36.8%) effect, sf: slope of PK/PD relationship

4.(ii).B Outline of the review by PMDA

4.(ii).B.(1) Relationship between serum eculizumab concentration and hemolytic activity

The applicant explained that eculizumab is effective at serum concentrations of ≥ 35 μg/mL and described its rationale as follows:

Eculizumab concentrations below 35 μg/mL tended to result in an increased level of hemolytic activity in a clinical study in patients with idiopathic membranous glomerulopathy (C99-004) and it was shown that ≥ 35 μg/mL of eculizumab blocked C5 (as assessed by measuring hemolytic activity) in ≥ 90% of blood samples obtained from all clinical studies conducted before Study C99-004 (Investigator's Brochure, Eculizumab. Version 11, 28 July 2005 [Alexion Pharmaceuticals Inc. In-house material]).

On the other hand, the serum concentrations of C5 in 7 normal volunteers were 99 to 134 μg/mL and the serum concentrations of C5 in 21 patients with diseases associated with C3 or C5 abnormalities were 86 to 122 μg/mL (*J Clin Invest*. 1977;59:704-715.). Eculizumab binds to C5, blocks its cleavage into C5a and C5b, and inhibits terminal complement activity. Theoretically, one molecule of eculizumab binds to two molecules of C5. Taking account of these findings, as 35 μg/mL of eculizumab (about 236 nM; molecular weight, about 148 kDa) is considered to bind to 95 μg/mL of C5 (about 500 nM; molecular weight, about 196 kDa) in human serum, a serum eculizumab concentration of "35 μg/mL" has been chosen as a threshold for efficacy.

The relationship between serum concentration and hemolytic activity in clinical studies in PNH patients (C02-001, TRIUMPH, SHEPHERD, C07-001) also showed that serum trough concentrations of ≥ 35 μg/mL are sufficient to maintain a hemolytic activity of ≤ 20% in the majority of patients (see Figure 2).

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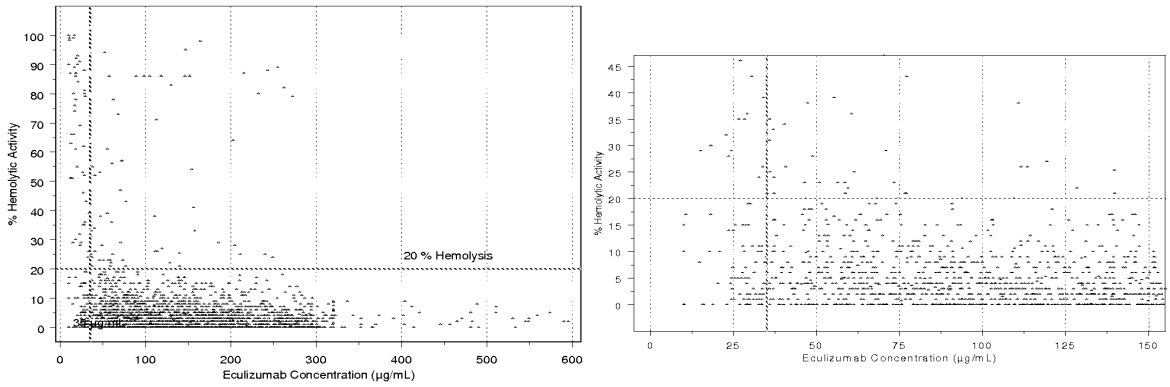

Figure 2. Relationship between serum eculizumab concentration and hemolytic activity in PNH patients (Left figure, All plots; Right figure, Magnification of the plots)

PMDA considers as follows:

With respect to the relationship between eculizumab serum concentration and hemolytic activity, as shown in Figure 2, the level of hemolytic activity was < 20% at serum concentrations of ≥ 35 μg/mL in the majority of samples (89.8% [1886 of 2101 samples]) while the level of hemolytic activity was ≥ 20% even at serum concentrations of ≥ 35 μg/mL in some patients (4.1% [87 of 2101 samples]). Therefore, serum concentrations of eculizumab ≥ "35 μg/mL" do not necessarily sufficiently inhibit hemolysis in all patients. However, taking account of the results from the TRIUMPH study, the efficacy of eculizumab can be expected at the proposed dosage regimen [see "4.(iii).B.(4) Efficacy" and "4.(iii).B.(7) Dosage and administration"].

4.(ii).B.(2) Pharmacokinetics/pharmacodynamics in Japan and overseas

The applicant explained about differences in the pharmacokinetics and pharmacodynamics (hemolytic activity) of eculizumab between Japan and overseas as follows:

Although the CL and Vc estimated from a PPK analysis in Japanese PNH patients are as shown in Table 6, when adjusted for body weight (mean ± SD), the CL was 0.282 ± 0.0870 mL/h/kg and the Vc was 101.9 ± 27.51 mL/kg, which were similar to the CL and Vc estimated from a PPK analysis in foreign PNH patients (see Table 8 and Table 10).

Observed serum concentrations of eculizumab over time in a Japanese clinical study (C07-001) and foreign clinical studies (C02-001, TRIUMPH, SHEPHERD) are as shown in Figure 3 and there were no particularly major differences.

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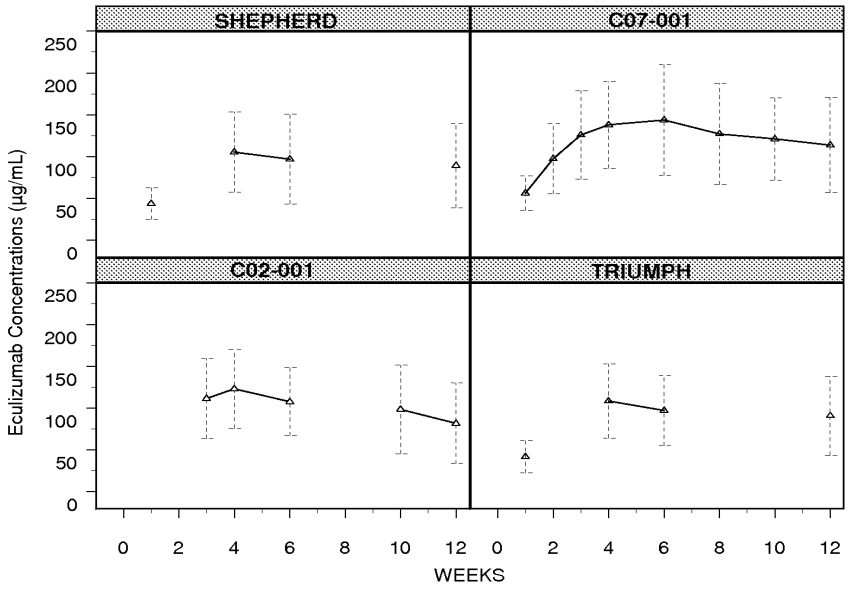

Figure 3. Serum eculizumab concentrations over time in clinical studies (C02-001, TRIUMPH, SHEPHERD, C07-001)

Furthermore, as to the pharmacodynamics of eculizumab, though figures showing the measured hemolytic activity over time in the Japanese clinical study (C07-001) and foreign clinical studies (TRIUMPH, SHEPHERD) are currently being prepared, the eculizumab pharmacodynamic parameters for hemolytic activity estimated using the cumulative Weibull function were similar between Japan and overseas (see Table 7 and Table 11).

The data from all Japanese and foreign studies in PNH patients (C02-001, TRIUMPH, SHEPHERD, E05-001, C07-001; a total of 177 patients) were pooled and the PK/PD relationship of eculizumab was investigated. As a result, although the statistically significant covariates were an effect of body weight on CL and Vc and an effect of gender on CL, there were no particular differences in the pharmacodynamic effect (hemolytic activity) except for Week 1. The CL of eculizumab (C02-001, TRIUMPH, SHEPHERD, and C07-001 were pooled; mean ± SD) was 0.0268 ± 0.0115 L/h in male patients and 0.0182 ± 0.0071 L/h in female patients and there were no major differences.

Based on the above, although the pharmacokinetics of eculizumab in Japanese and foreign PNH patients may be slightly different due to the effect of body weight, the pharmacodynamic effect is similar and there are no particular differences in the safety profile of eculizumab between Japan and overseas. Therefore, no dose adjustment should be required for Japanese PNH patients.

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PMDA considers as follows:
Concerning the pharmacokinetics and pharmacodynamics of eculizumab in Japan and overseas, the observed pharmacokinetic data were compared, PPK analysis was performed and the pharmacodynamic parameters were estimated using the cumulative Weibull function. As a result, since blood trough concentrations over time were slightly higher in Japanese PNH patients with lower body weight compared with foreign PNH patients, body weight may affect eculizumab blood concentrations over time. However, though the results of the pharmacodynamic hemolytic assay from Japanese and foreign clinical studies are currently being requested, there were no particular differences in the pharmacodynamic parameters (hemolytic activity) estimated using the cumulative Weibull function between Japan and overseas and there was also little difference in the CL value estimated from a PPK analysis between Japan and overseas. Therefore, differences in the pharmacokinetics of eculizumab due to the effect of body weight are unlikely to significantly affect inhibition of hemolysis by eculizumab.

The above analysis indicated the possibility that body weight affects the pharmacokinetics of eculizumab and safety data from Japanese and foreign clinical studies (TRIUMPH, SHEPHERD, C07-001) showed higher incidences of nasopharyngitis, headache, and eczema, etc. in Japanese PNH patients than in foreign PNH patients [see "4.(iii).B.(5) Safety"]. As Japanese patients tend to have lower body weight than foreign patients, PMDA is currently asking the applicant about the effects of body weight on the safety and efficacy of eculizumab.

4.(ii).B.(3) HAHA induction and its impact on the pharmacokinetics of eculizumab
Based on the results from Japanese and foreign clinical studies of eculizumab, the applicant explained the impact of HAHA induction on the pharmacokinetics of eculizumab as follows:
A total of 940 patients including non-PNH patients were treated with eculizumab in Japanese and foreign clinical studies. There was no apparent impact of HAHA induction on the pharmacokinetic parameters of eculizumab.

Among PNH clinical studies, there were no patients with a positive HAHA response in Studies C02-001, E02-001, and X03-001. Although 1 patient in the placebo group and 1 patient in the eculizumab group had a positive HAHA response in the TRIUMPH study and 2 patients had a positive HAHA response in the SHEPHARD study, there was no apparent impact on the pharmacokinetics of eculizumab. In Study C07-001, there were 3 patients with positive HAHA response before eculizumab treatment, but none after eculizumab treatment. No analysis has been performed for Study E05-001.

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PMDA considers as follows:

An assay method to detect HAHA used in studies other than the Japanese clinical study (C07-001) was the method before improvement. Even in the Japanese clinical study (C07-001), a positive HAHA response was detected before eculizumab treatment and the assay may need further improvement. Furthermore, the number of patients who developed HAHA in clinical studies was limited. Therefore, the impact of HAHA induction on the pharmacokinetics of eculizumab is undefined at present. It is desirable to further increase the assay sensitivity to HAHA and establish an assay for the measurement of neutralizing HAHA to eculizumab. After the market launch, if adverse events of infusion reaction etc. which are considered associated with HAHA induction are reported or there is loss of efficacy etc., the HAHA assay should be performed as appropriate and information on HAHA should be collected and provided to the medical practice [see "4.(iii).B.(5).7) Immunogenicity and human anti-human antibodies (HAHA)"].

4.(iii) Summary of clinical efficacy and safety

4.(iii).A Summary of the submitted data

A total of 8 clinical studies presented in the following Table 12 were submitted as evaluation data.

Table 12. Listing of PNH clinical studies submitted

	Study Number	Phase	Primary objective of study	Design	N	Duration of treatment	Primary efficacy endpoint
Japan	C07-001	II	Efficacy, safety, pharmacokinetics, and pharmacodynamics	Open-label, uncontrolled study	29	12 weeks	Change of LDH
	E07-001 (Interim report)	II	Efficacy, safety, pharmacokinetics, and pharmacodynamics	Open-label, uncontrolled study (C07-001 extension)	27	26 weeks	–
Overseas	C02-001	I	Efficacy, safety, pharmacokinetics, and pharmacodynamics	Open-label, uncontrolled study	11	12 weeks	–
	E02-001	I	Safety	Open-label, uncontrolled study (C02-001 extension)	11	52 weeks	–
	X03-001/ X03-001A	II	Safety	Open-label, uncontrolled study (E02-001 extension)	11/10	A total of 104 weeks	–
	C04-001 (TRIUMPH)	III	Efficacy and safety	Placebo-controlled, double-blind, comparative study	43 (eculizumab group) 44 (placebo group)	26 weeks	Hb stabilization, PRBC units transfused
	C04-002 (SHEPHERD)	III	Efficacy and safety	Open-label, uncontrolled study	97	52 weeks	LDH AUC
	E05-001 (Interim report)	III	Safety	Open-label, uncontrolled study (X03-001, C04-001, and C04-002 extension)	187	Up to 2 years	–

LDH: lactate dehydrogenase, PRBC: packed red blood cell

4.(iii).A.(1) Japanese phase II clinical study (5.3.5.2.6, Study C07-001 [[redacted] to [redacted]])

A multicenter, open-label, uncontrolled study was conducted at 9 centers in Japan to evaluate the efficacy, safety, pharmacokinetics, and pharmacodynamics of eculizumab in PNH patients aged ≥ 12 years having a GPI-deficient RBC clone (PNH type III) ≥ 10% (Target sample size of 25).

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Eculizumab was to be administered by intravenous infusion over 25 to 45 minutes at a dose of 600 mg weekly for a total of 4 doses followed 1 week later by 900 mg every 2 weeks for a total of 5 doses (the duration of treatment was 12 weeks). Patients were to receive a meningococcal vaccine (serogroups "A, C, Y, and W135") at least 2 weeks prior to treatment with eculizumab.

All of 29 treated patients were included in the per protocol set (PPS) and in the efficacy, safety, pharmacokinetic, and pharmacodynamic analyses. Two patients were discontinued from the study after 10 weeks of treatment due to lack of efficacy.

The primary efficacy endpoint was the change of lactate dehydrogenase (LDH) from baseline[††] to Week 12. There was a significant reduction in LDH from baseline (median [min., max.], 1814.0 units/L (U/L) [627.8 U/L, 3642.5 U/L]) to Week 12[‡‡] (244.0 U/L [187.0 U/L, 2715.0 U/L]) ($P < 0.0001$, Wilcoxon signed-rank test).

Regarding safety, the incidence of adverse events was 96.6% (28 of 29 patients) and the incidence of adverse events for which a causal relationship to study drug could not be denied (adverse drug reactions) was 93.1% (27 of 29 patients). Adverse events occurring in at least 10.0% of patients were headache (51.7% [15 of 29 patients]), nasopharyngitis (41.4% [12 of 29 patients]), nausea (20.7% [6 of 29 patients]), diarrhoea (13.8% [4 of 29 patients]), vomiting (10.3% [3 of 29 patients]), pyrexia (10.3% [3 of 29 patients]), and eczema (10.3% [3 of 29 patients]) and adverse drug reactions occurring in at least 10.0% of patients were headache (51.7% [15 of 29 patients]), nasopharyngitis (37.9% [11 of 29 patients]), nausea (20.7% [6 of 29 patients]), vomiting (10.3% [3 of 29 patients]), pyrexia (10.3% [3 of 29 patients]), and eczema (10.3% [3 of 29 patients]).

No deaths occurred. Serious adverse events of "pyrexia and CRP increased" were reported by 1 patient and their causal relationship to study drug could not be denied.

4.(iii).A.(2) Japanese phase II clinical study (5.3.5.2.7, Study E07-001 [20 to 20 (Interim report)])

A multicenter, open-label, uncontrolled study was conducted at 9 centers in Japan to evaluate the safety, efficacy, pharmacokinetics, and pharmacodynamics of eculizumab in PNH patients who completed Study C07-001 (Target number of patients of 27).

Eculizumab was to be administered by intravenous infusion over 25 to 45 minutes at a dose of 900 mg every 2 weeks for 52 weeks (The interim report contains data up to Week 26).

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†† Mean LDH over the 4 weeks prior to start of treatment
‡‡ Missing data were imputed using last observation carried forward (LOCF).

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All of 27 treated patients were included in the PPS and in the efficacy, safety, pharmacokinetic, and pharmacodynamic analyses. One patient was discontinued from the study at Week 18 due to patient request.

Regarding safety, the incidence of adverse events was 100.0% (27 of 27 patients) and the incidence of adverse drug reactions was 92.6% (25 of 27 patients). Adverse events occurring in at least 10.0% of patients were nasopharyngitis (51.9% [14 of 27 patients]), headache (18.5% [5 of 27 patients]), blood ALP increased (18.5% [5 of 27 patients]), and anaemia (11.1% [3 of 27 patients]) and adverse drug reactions occurring in at least 10.0% of patients were nasopharyngitis (51.9% [14 of 27 patients]), headache (18.5% [5 of 27 patients]), and blood ALP increased (18.5% [5 of 27 patients]).

No deaths occurred. Serious adverse events reported were "sepsis, cellulitis, skin disorder, and acute renal failure" in 1 patient, "anaemia" in 1 patient, "pyrexia and herpes simplex infection" in 1 patient, and "bronchitis" in 1 patient and a causal relationship to study drug could not be denied for all events except for anaemia.

Efficacy, pharmacokinetic, and pharmacodynamic evaluations are not planned for the interim report and the final report will be complete in June 2011.

4.(iii).A.(3) Foreign phase I clinical study (5.3.5.2.1, Study C02-001 [May 2002 to January 2003]; *N Engl J Med*. 2004;350:552-558.)

A multicenter, open-label, uncontrolled study was conducted at 2 centers overseas to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of eculizumab in PNH patients who were at least 18 years of age and had a GPI-deficient RBC clone (PNH type III) ≥ 10% (Target number of patients of 10).

Eculizumab was to be administered by intravenous infusion over at least 30 minutes at a dose of 600 mg weekly for a total of 4 doses followed 1 week later by 900 mg every 2 weeks for a total of 4 doses (the duration of treatment was 10 weeks). Patients received a meningococcal vaccine (serogroups "A and C") at least 2 weeks prior to treatment with eculizumab.

All of 11 treated patients were included in the PPS and in the safety, pharmacokinetic, and pharmacodynamic analyses. None of the patients was discontinued from the study.

Regarding safety,[§§] the incidence of adverse events was 100.0% (11 of 11 patients). Adverse events occurring in at least 2 patients were headache and upper respiratory tract infection (3 patients each) and

[§§] The causal relationship of an adverse event to study drug was assessed on a 3-point scale (unlikely, possible, probable).

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dizziness, nasal congestion, chest pain, influenza-like symptoms, chills, insomnia, arthralgia, and nausea (2 patients each).

No deaths occurred. Serious adverse events reported were "chest infection" in 1 patient and "nausea, vomiting, headache, dizziness, and shivering" in 1 patient.

4.(iii).A.(4) Foreign phase I clinical study (5.3.5.2.2, Study E02-001 [August 2002 to December 2003]; *Blood*. 2007;110:4123-4128, *Blood*. 2005;106:2559-2565.)

A multicenter, open-label, uncontrolled study was conducted at 2 centers overseas to evaluate the safety and tolerability of eculizumab in PNH patients who completed Study C02-001 (Target number of patients of 11).

Eculizumab was to be administered by intravenous infusion over at least 30 minutes at a dose of 900 mg every 2 weeks for 52 weeks.

All of 11 treated patients were included in the safety analysis population. None of the patients was discontinued from the study.

Regarding safety,*** the incidence of adverse events was 100.0% (11 of 11 patients). Adverse events occurring in at least 2 patients are presented in Table 13.

Table 13. Adverse events occurring in at least 2 patients

Adverse event	Incidence	N	Adverse event	Incidence	N	Adverse event	Incidence	N
Overall	100.0%	11	Abdominal pain	18.2%	2	Myalgia	18.2%	2
Throat sore	36.4%	4	Constipation	18.2%	2	Nasal congestion	18.2%	2
Influenza-like symptoms	36.4%	4	Diarrhoea	18.2%	2	Loose stools	18.2%	2
Upper respiratory tract infection	27.3%	3	Feeling cold	18.2%	2	Throat pain	18.2%	2
Nausea	27.3%	3	Herpes simplex	18.2%	2	Throat tightness	18.2%	2
Contusion	27.3%	3	Inflicted injury	18.2%	2	Fatigue	18.2%	2
Cough	27.3%	3	Lethargy	18.2%	2	Vomiting	18.2%	2
Pain	27.3%	3	Lower respiratory tract infection	18.2%	2			

n = 11

No deaths occurred. Serious adverse events of "extravascular haemolysis, viral syndrome, and neutropenia" were reported by 1 patient.

4.(iii).A.(5) Foreign phase II clinical studies (5.3.5.2.3, Studies X03-001 and X03-001A [August 2003 to December 2005]; *Haematologica*. 2005;90:ECR40.)

Study X03-001 was a multicenter, open-label, uncontrolled study conducted at 2 centers overseas to evaluate the safety and tolerability of eculizumab in PNH patients who completed Study E02-001. PNH

*** The causal relationship of an adverse event to study drug was assessed on a 3-point scale (unlikely, possible, probable).

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patients who completed Study X03-001 were to participate in Study X03-001A.

Eculizumab was to be administered by intravenous infusion over at least 30 minutes at a dose of 900 mg every 2 weeks for a total of 104 weeks (52 weeks for Study X03-001; 52 weeks for Study X03-001A). Patients received a meningococcal vaccine (serogroups "C" or "A, C, Y, and W135").

A total of 11 treated patients in Study X03-001 and a total of 10 treated patients in Study X03-001A were included in the safety analysis. One patient was discontinued from Study X03-001 at about Week 38 due to patient request. None of the patients was discontinued from Study X03-001A.

Regarding safety,[†††] the incidence of adverse events was 100.0% (11 of 11 patients) in Study X03-001 and the incidence of adverse events was 90.0% (9 of 10 patients) and the incidence of adverse drug reactions was 20.0% (2 of 10 patients) in Study X03-001A. Adverse events occurring in at least 2 patients in Study X03-001 are presented in Table 14.

Table 14. Adverse events occurring in at least 2 patients in Study X03-001

Adverse event	Incidence	N	Adverse event	Incidence	N	Adverse event	Incidence	N
Overall	100.0%	11	Headache	27.3%	3	Viral infection	18.2%	2
Fatigue	45.5%	5	Dyspnoea	27.3%	3	Contusion	18.2%	2
Lower respiratory tract infection	36.4%	4	Nasal congestion	27.3%	3	Arthralgia	18.2%	2
Pain in extremity	36.4%	4	Abdominal pain	18.2%	2	Dizziness	18.2%	2
Pharyngolaryngeal pain	36.4%	4	Nausea	18.2%	2	Insomnia	18.2%	2
Abdominal discomfort	27.3%	3	Lethargy	18.2%	2	Epistaxis	18.2%	2
Diarrhoea	27.3%	3	Cystitis	18.2%	2	Rhinitis	18.2%	2
Influenza	27.3%	3	Upper respiratory tract infection	18.2%	2	Hypertension	18.2%	2
Nasopharyngitis	27.3%	3						

n = 11

Adverse events occurring in at least 2 patients in Study X03-001A were pharyngolaryngeal pain (40.0% [4 of 10 patients]), nasopharyngitis (30.0% [3 of 10 patients]), pyrexia (20.0% [2 of 10 patients]), lower respiratory tract infection (20.0% [2 of 10 patients]), and viral infection (20.0% [2 of 10 patients]) and there were no adverse drug reactions reported by ≥ 2 patients.

No deaths occurred. Serious adverse events reported were "abscess limb and infected left thumb" in 1 patient in Study X03-001 and "pyrexia" in 1 patient, "viral infection," in 1 patient, and "urinary tract infection" in 1 patient in Study X03-001A and a causal relationship to study drug was denied only for "viral infection" observed in Study X03-001A.

[†††] While the causal relationship of an adverse event to study drug was assessed on a 3-point scale (unlikely, possible, probable) in Study X03-001, the causal relationship of an adverse event to study drug was assessed on a 4-point scale (unrelated, possible, probable, definite) in Study X03-001A.

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4.(iii).A.(6) Foreign phase III clinical study (5.3.5.1.1, Study C04-001 [TRIUMPH] [August 2004 to December 2005]; *Br J Haematol.* 2008;142:263-272, *N Engl J Med.* 2006;355:1233-1243.)

A multicenter, randomized, placebo-controlled, double-blind, comparative study was conducted at 34 centers overseas to evaluate the efficacy and safety of eculizumab in PNH patients who were at least 18 years of age and had a GPI-deficient RBC clone (PNH type III) ≥ 10% (Target number of patients of 75).

Eculizumab or placebo was to be administered by intravenous infusion over 25 to 45 minutes at a dose of 600 mg weekly for a total of 4 doses, followed by 900 mg 1 week later, and then 900 mg every 2 weeks for a total of 26 weeks. Patients received a meningococcal vaccine (serogroups "C," "A and C" or "A, C, Y, and W135") at least 2 weeks prior to treatment with eculizumab or placebo.

All of 87 treated patients (44 patients in the placebo group, 43 patients in the eculizumab group) were included in the intent to treat population and in the efficacy and safety analyses. Two patients in the eculizumab group were discontinued from the study due to an adverse event or patient request and 10 patients in the placebo group were discontinued from the study due to lack of efficacy.

The co-primary efficacy endpoints were Hb stabilization[‡‡‡] and the number of packed red blood cell (PRBC) units transfused. Hb stabilization was achieved in 0.0% of the placebo group (0 of 44 patients) compared with 48.8% of the eculizumab group (21 of 43 patients) and there was a significant difference between the groups ($P < 0.001$, Fisher's exact test). Ten units [2 units, 21 units] (median [min., max.]) of PRBCs were administered in the placebo group compared with 0 units [0 unit, 16 units] in the eculizumab group and there was a significant difference between the groups ($P < 0.001$, Wilcoxon's rank sum test).

Regarding safety, adverse events occurred in 90.9% of the placebo group (40 of 44 patients) and 100.0% of the eculizumab group (43 of 43 patients). Adverse drug reactions occurred in 25.0% of the placebo group (11 of 44 patients) and 55.8% of the eculizumab group (24 of 43 patients). Adverse events occurring in at least 10.0% of patients in either group are presented in Table 15. Adverse drug reactions occurring in at least 10.0% of patients in either group were headache only and the incidence of headache as an adverse drug reaction was 4.5% (2 of 44 patients) in the placebo group and 32.6% (14 of 43 patients) in the eculizumab group.

‡‡‡ Each patient underwent an initial observation period to identify the Hb concentration that necessitated RBC transfusion (the set-point). To achieve Hb stabilization, a patient had to maintain an Hb concentration above the Hb set-point and avoid any RBC transfusion for the entire treatment period.

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Table 15. Adverse events occurring in at least 10.0% of patients in either group

Adverse event	Placebo (N = 44)		Eculizumab (N = 43)	
	Incidence	N	Incidence	N
Overall	90.9%	40	100.0%	43
Headache	27.3%	12	44.2%	19
Nasopharyngitis	18.2%	8	23.3%	10
Back pain	9.1%	4	18.6%	8
Nausea	11.4%	5	16.3%	7
Upper respiratory tract infection	22.7%	10	14.0%	6
Cough	9.1%	4	11.6%	5
Fatigue	2.3%	1	11.6%	5
Diarrhoea	11.4%	5	9.3%	4
Arthralgia	11.4%	5	7.0%	3
Abdominal pain	11.4%	5	4.7%	2
Dizziness	11.4%	5	4.7%	2
Vomiting	11.4%	5	4.7%	2
Viral infection	11.4%	5	2.3%	1

No deaths occurred. Serious adverse events occurred in 20.5% of the placebo group (9 of 44 patients) and 9.3% of the eculizumab group (4 of 43 patients), which included "exacerbation of PNH" (3 patients) and "urinary tract infection and central line infection," "exacerbation of PNH, anaemia, and pyrexia," "neutropenia," "neutropenia, cellulitis, and folliculitis," "haemolysis and upper respiratory tract infection," and "viral infection" (1 patient each) in the placebo group and "exacerbation of PNH," "renal colic," "prolapsed lumbosacral disc," and "α-Streptococcal bacteraemia" (1 patient each) in the eculizumab group and a causal relationship to study drug could not be denied only for pyrexia in the placebo group.

4.(iii).A.(7) Foreign phase III clinical study (5.3.5.2.4, Study C04-002 [SHEPHERD] [December 2004 to October 2006]; *Blood*. 2008;111:1840-1847, *Br J Haematol*. 2008;142:263-272.)

A multicenter, open-label, uncontrolled study was conducted at 33 centers overseas to evaluate the safety and efficacy of eculizumab in PNH patients who were at least 18 years of age and had a GPI-deficient RBC clone (PNH type III) ≥ 10% (Target number of patients of 85).

Eculizumab was to be administered by intravenous infusion over 25 to 45 minutes at a dose of 600 mg weekly for a total of 4 doses, followed by 900 mg 1 week later, and then 900 mg every 2 weeks for a total of 52 weeks. Patients received a meningococcal vaccine (serogroups "C," "A and C," or "A, C, Y, and W135") at least 2 weeks prior to treatment with eculizumab.

All of 97 treated patients were included in the PPS and in the efficacy and safety analyses. One patient was discontinued from the study because the patient died due to an adverse event (brain herniation) at Week 4.

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The primary efficacy endpoint was LDH area under the curve (AUC) from baseline to Week 52 (LDH AUC) and the change in the LDH AUC[§§§] (median [min., max.]) was -632263.5 U/L × day [-1788823.5 U/L × day, -74498.0 U/L × day], which was significantly different from zero ($P < 0.001$, Wilcoxon signed rank test).

Regarding safety, the incidence of adverse events was 97.9% (95 of 97 patients) and the incidence of adverse drug reactions was 64.9% (63 of 97 patients). Adverse events occurring in at least 10.0% of patients are presented in Table 16. Adverse drug reactions occurring in at least 10.0% of patients were headache only (42.3% [41 of 97 patients]).

Table 16. Adverse events occurring in at least 10.0% of patients

Adverse event	Incidence	N	Adverse event	Incidence	N	Adverse event	Incidence	N
Overall	97.9%	95	Pyrexia	19.6%	19	Arthralgia	12.4%	12
Headache	52.6%	51	Back pain	15.5%	15	Abdominal pain	11.3%	11
Nasopharyngitis	32.0%	31	Dizziness	14.4%	14	Myalgia	10.3%	10
Upper respiratory tract infection	29.9%	29	Urinary tract infection	13.4%	13	Vomiting	10.3%	10
Nausea	20.6%	20	Diarrhoea	12.4%	12			

n = 97

One patient with "brain herniation" died, but its causal relationship to study drug was denied. The incidence of serious adverse events was 19.6% (19 of 97 patients) and serious adverse events occurring in at least 2 patients were anaemia (4 patients), pyrexia (3 patients), haemolysis (2 patients), and headache (2 patients). Among the observed serious adverse events, a causal relationship to study drug could not be denied for pyrexia (2 patients) and abdominal distension, renal impairment, headache, anxiety, cholangitis, and viral infection (1 patient each).

4.(iii).A.(8) Foreign phase III clinical study (5.3.5.2.5, Study E05-001 [May 2005 to March 2006 (Interim report)]; *Blood*. 2007;110:4123-4128.)

An open-label, uncontrolled study was conducted at 33 centers overseas to evaluate the safety of eculizumab in PNH patients who completed the TRIUMPH study, the SHEPHERD study, or Study X03-001A[****] (Target number of patients was not specified [up to 170 patients]).

Patients who completed the SHEPHERD study or Study X03-001 were to receive 900 mg of eculizumab every 2 weeks via 25 to 45 minute intravenous infusion. On the other hand, study drug was to be administered by intravenous infusion over 25 to 45 minutes to patients who completed the TRIUMPH study as follows: placebo-treated patients were to receive 600 mg of blinded eculizumab weekly for a total of 4 doses followed 1 week later by 900 mg of open-label eculizumab every 2 weeks; and eculizumab-treated patients were to receive 900 mg of blinded eculizumab in Week 1 and Week 3 and blinded placebo in Week 2 and Week 4 followed 1 week later by 900 mg of open-label eculizumab

§§§ The LDH AUC from baseline to 52 weeks calculated by the trapezoidal rule minus the baseline LDH AUC calculated by assuming no change in LDH from baseline through 52 weeks

**** TRIUMPH patients who had discontinued receiving study drug before Week 26 were also eligible, if their data up to Week 26 had been evaluated for efficacy and safety.

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every 2 weeks. The duration of treatment was up to 2 years. Patients received a meningococcal vaccine (serogroups "A and C" or "A, C, Y, and W135") as appropriate.

All of 96 treated patients were included in the PPS and in the safety analysis (at the time of interim analysis in March 2006, the mean duration of treatment was 5.7 ± 1.9 months). None of the patients was discontinued from the study.

Regarding safety, the incidence of adverse events was 89.6% (86 of 96 patients) and the incidence of adverse drug reactions was 38.5% (37 of 96 patients). Adverse events occurring in at least 10.0% of patients are presented in Table 17. Adverse drug reactions occurring in at least 10.0% of patients were headache only (14.5% [14 of 96 patients]).

Table 17. Adverse events occurring in at least 10.0% of patients

Adverse event	Incidence	N	Adverse event	Incidence	N	Adverse event	Incidence	N
Overall	89.6%	86	Upper respiratory tract infection	15.6%	15	Cough	10.4%	10
Headache	30.2%	29	Diarrhoea	12.5%	12	Nausea	10.4%	10
Nasopharyngitis	30.2%	29	Arthralgia	10.4%	10			

No deaths occurred. The incidence of serious adverse events was 9.4% (9 of 96 patients) and serious adverse events occurring in at least 2 patients were cellulitis (2 patients), infection (2 patients), and viral infection (2 patients). Among the observed serious adverse events, a causal relationship to study drug could not be denied for cellulitis, infection, viral infection, haemophilus infection, septic shock, and headache (1 patient each).

The final report will be submitted in the [redacted] quarter of 20[redacted].

4.(iii).B Outline of the review by PMDA

PMDA's review focused on the following points.

4.(iii).B.(1) Clinical positioning of eculizumab in PNH treatment

The applicant explained the pathology and treatment of PNH and the clinical positioning of eculizumab as follows:

It has been reported that in PNH patients, hemolysis results in symptoms of anaemia requiring blood transfusions, fatigue, hemoglobinuria, and recurrent abdominal pain, etc. and furthermore, serious events associated with pulmonary hypertension, renal failure, and thromboembolism (TE) occur (*Blood*. 2007;110:4123-4128, *JAMA*. 2005;293:1653-1662, *Blood*. 1981;57:83-89.). Patients with aplastic anemia (AA) or myelodysplastic syndromes (MDS) may go on to develop PNH and PNH patients often have AA or MDS as well.

Currently, the following therapies are used for PNH patients with clinically relevant hemolysis to relieve clinical symptoms considered associated with chronic intravascular hemolysis (anaemia, fatigue,

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abdominal pain, TE, renal failure, pulmonary hypertension, etc.): (1) iron preparation/folic acid, (2) erythropoietin, (3) blood transfusion, (4) haptoglobin preparation, (5) corticosteroids, (6) anabolic steroids, (7) anticoagulants, (8) allogeneic hematopoietic stem cell transplantation, etc. However, the therapies (1) to (7) do not inhibit hemolysis itself. Allogeneic hematopoietic stem cell transplantation is the curative therapy for PNH, but is indicated for PNH patients with severe bone marrow failure only. Reports on the outcome of allogeneic hematopoietic stem cell transplants for PNH are shown in Table 18.

Table 18. The outcome of allogeneic hematopoietic stem cell transplants for PNH

Publication	No. of patients	Age (Median)	Mortality
Br J Haematol. 1999;104:392-396.	57	28	44% after 2 years[b]
Biol Blood Marrow Transplant. 2003;9:689-697.	7	34	43% at about 2-year follow-up
Blood. 2005;106:3699-3709.[a]	121	30	44% after 10 years
Thomas' Hematopoietic Cell transplantation. 3rd ed.; 2007:1002-1006.	23	29	39% (the duration of follow-up is unknown)

[a] The supplemental document link at the top of the online article, at the Blood website
[b] Outcome of 48 recipients of HLA-identical sibling bone marrow transplants

Since PNH patients with AA or MDS also have bone marrow failure, basically, AA or MDS is also treated simultaneously (immunosuppressants, blood transfusion, allogeneic hematopoietic stem cell transplantation, etc.).

On the other hand, since the main clinical studies of eculizumab (TRIUMPH, SHEPHERD, C07-001) have demonstrated that eculizumab reduces hemolysis, eculizumab is positioned as a first-choice drug for PNH patients. As allogeneic hematopoietic stem cell transplantation is associated with high rates of transplant-related complications and mortality, eculizumab should be considered first for PNH patients with severe bone marrow failure as well.

PMDA considers as follows:
There is no effective existing therapy for the hemolysis of PNH at present. Since the TRIUMPH study demonstrated that eculizumab stabilized Hb levels and reduced transfusion requirements by reducing hemolysis [see "4.(iii).B.(4) Efficacy"], if appropriate patients are selected, eculizumab will become an effective therapy for the hemolysis of PNH. Although allogeneic hematopoietic stem cell transplantation can be a curative therapy for PNH, not every patient can find a suitable donor and the rates of transplant-related complications and mortality are high. Thus, the decision of whether or not to perform allogeneic hematopoietic stem cell transplantation should be carefully considered, taking account of the bone marrow function and age of the patient, etc. It is understood that eculizumab may be used first also for candidates for allogeneic hematopoietic stem cell transplantation.

Meanwhile, eculizumab is not a curative therapy for PNH and continued treatment is needed to inhibit hemolysis. By protecting PNH type III RBCs against hemolysis, the percentage of PNH type III RBCs in blood increases in patients being treated with eculizumab and serious hemolysis may be induced if

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eculizumab is discontinued [see "4.(iii).B.(5).8) Safety after eculizumab discontinuation"]. The effects of hemolysis inhibition by eculizumab on the development of TE and survival prognosis are unclear and moreover, eculizumab may increase the risk of infections with *Neisseria meningitidis* etc. [see "4.(iii).B.(5).4) Meningococcal infections" and "4.(iii).B.(5).5) Other infections"]. Therefore, prior to the initiation of eculizumab treatment, patients should be fully assessed for the severity of hemolysis or anaemia and complications etc. and then, eculizumab should be used only when the clinical benefits of eculizumab can be reasonably expected to outweigh the possible risks [see "4.(iii).B.(6) Indication" for the intended population].

4.(iii).B.(2) Differences in PNH between Japan and overseas

The results of a comparison of the epidemiology of PNH between Japan and the US based on the publication (*Medicine*. 2004;83:193-207.) are shown in Table 19.

Table 19. Comparison of the epidemiology of PNH between Japan and the US

		Japan	US
Gender difference in prevalence (Male/Female)		1.3	0.8
Age of onset (Median [min., max.])		45 years [10 years, 86 years]	30 years [4 years, 80 years]
Survival time after diagnosis (Median)		25.0 years	23.3 years
Preceding AA or MDS (%)	AA	37.8%	29.0%
	MDS	4.8%	5.1%
Initial symptoms (%)	Hemoglobinuria	33.5%	50.0%
	Anaemia	94.3%	88.1%
	Leukopenia/Neutropenia	7.23%	45.5%
	Thrombocytopenia	63.2%	52.3%
	Infection	3.4%	13.6%
	TE	6.2%	19.3%
Laboratory findings (Mean ± SE)	Hb level	8.2 ± 0.2 g/dL	9.7 ± 0.2 g/dL
	Reticulocyte count	78.3 ± 6.2×10^6/L	195.3 ± 13.1×10^6/L
	White blood cell count	3475.3 ± 137.5×10^6/L	4947 ± 198.6×10^6/L
	Neutrophil count	1781.6 ± 132.5×10^6/L	3005.1 ± 156.4×10^6/L
	Platelet count	96.0 ± 5.8×10^6/L	140.1 ± 8.6×10^6/L
	LDH level	1572.3 ± 91.7 U/L	2337.2 ± 405.6 U/L
Complication (%)	TE	4.3%	31.8%
	Severe infection	9.1%	18.2%
	Hematopoietic failure	36.4%	33.0%
	MDS	3.8%	3.4%
	Leukemia	2.9%	0.6%
	Renal failure	10.5%	9.1%
Cause of death (%)	TE	7.9%	42.1%
	Severe infection	36.8%	36.8%
	Renal failure	18.4%	7.9%
	Haemorrhage	23.7%	10.5%
	MDS/Leukemia	15.8%	7.9%
	Cancer	5.3%	5.3%
	Unknown	0.0%	5.3%
Treatment (%)	No treatment	28.2%	5.7%
	Blood transfusion	40.2%	61.9%
	Anabolic steroids	39.7%	36.9%
	Steroids	46.9%	82.4%
	ATG/ALG	2.9%	15.3%
	Cyclosporine	8.1%	9.7%
	Erythropoietin	3.3%	6.8%
	G-CSF	7.7%	1.7%
	Anticoagulants	4.3%	26.7%
	Allogeneic hematopoietic stem cell transplantation	1.9%	8.0%

ATG: antithymocyte globulin, ALG: antilymphocyte globulin, G-CSF: granulocyte-colony stimulating factor

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It has been reported that there were marked differences in the prevalences of TE and severe infection as complications between Japan and the US and the causes of death were also different, i.e. the percentages of patients who died from TE, renal failure, or haemorrhage etc. were different.

PMDA considers that as the results of this epidemiological study have indicated differences in complications and causes of death etc. between Japan and overseas, these differences should be noted when evaluating foreign clinical study data [see "4.(iii).B.(4) Efficacy" and "4.(iii).B.(5) Safety"].

4.(iii).B.(3) Data subject to evaluation

PMDA planned its review for eculizumab as follows:

For the clinical data submitted in the application, a confirmatory study in Japanese PNH patients has not been conducted. The estimated number of PNH patients in Japan is approximately 430 (Health Science Research Grants, Research Project on Intractable Diseases, Research Committee for determining the epidemiology of diseases not covered by Research Project on Treatment of Intractable Diseases, 1999 Research Results, 2000) and it is understood that it is difficult to conduct a confirmatory, double-blind comparative study in Japan alone. PNH is an acquired genetic disorder characterized by intravascular hemolysis due to a deficiency of the GPI-anchored terminal complement inhibitor CD59 and eculizumab is an anti-C5 antibody and reduces hemolysis by inhibiting C5 and blocking the formation of the terminal complement complex C5b-9. It is inferred from the pathology of the disease and the mechanism of action that eculizumab is insensitive to ethnic factors and is expected to have similar effects also in Japanese PNH patients.

Therefore, PMDA decided to position the TRIUMPH study, which is a foreign randomized, placebo-controlled, double-blind, comparative study, as the pivotal study and use the SHEPHERD study, which is an open-label, uncontrolled, phase III study enrolling a broader range of PNH patients than in the TRIUMPH study and Study C07-001, which is a Japanese open-label, uncontrolled, phase II study, as supporting efficacy and safety data, for evaluations. However, when evaluating the efficacy and safety of eculizumab in Japanese patients by comparing the results from the Japanese clinical study (C07-001) with the results from the foreign clinical studies (TRIUMPH and SHEPHERD), epidemiological differences in PNH [see "4.(iii).B.(2) Differences in PNH between Japan and overseas"] and differences in patient background etc. among the studies need to be fully taken into consideration and the following reviews were conducted [see "4.(iii).B.(4) Efficacy" and "4.(iii).B.(5) Safety"].

4.(iii).B.(4) Efficacy

As a result of the following reviews, PMDA considers that although the effects of eculizumab on the development of TE, which is a cause of death and is regarded as a serious problem in PNH patients, and survival prognosis etc. are undefined, as eculizumab has been shown to reduce hemolysis, if appropriate

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patients are selected, the clinical benefits of inhibition of decrease in Hb levels and reductions in transfusion requirements resulting from hemolysis inhibition by eculizumab can be expected. A final conclusion on the efficacy of eculizumab will be made, taking account of comments from the Expert Discussion.

4.(iii).B.(4).1) Efficacy endpoints

As the primary endpoints for the main clinical studies, "Hb stabilization" and "the number of PRBC units transfused" for the TRIUMPH study, "LDH AUC" from baseline to Week 52 for the SHEPHERD study, and "change of LDH" from baseline to Week 12 for Study C07-001 were chosen.

PMDA asked the applicant to provide a rationale for the primary endpoint for each study and explain the relationship between LDH, which was used as a primary endpoint measure for the SHEPHERD study and Study C07-001, and hemolysis inhibition, taking also into account that LDH can be a non-specific marker.

The applicant responded as follows:

Hemolysis is the underlying cause of clinical symptoms of PNH and it was decided to choose an objective measure of eculizumab-induced reduction of hemolysis as the primary endpoint. Achievement of "Hb stabilization" was defined as maintenance of an Hb concentration above the Hb set-point and avoidance of any RBC transfusion for the entire treatment period (each patient underwent an initial observation period to identify the Hb concentration that necessitated RBC transfusion [the set-point]). This is considered a measure of stable anemia. "The number of PRBC units transfused" is considered a measure that can assess the need for blood transfusion objectively to some extent, because patients received transfusion if Hb was ≤ 9.0 g/dL with clinical symptoms or if Hb was ≤ 7.0 g/dL without clinical symptoms in the TRIUMPH study.

On the other hand, LDH can be used as a marker for intravascular hemolysis because LDH is released into the bloodstream during the hemolysis of erythrocytes and LDH is considered a measure that can assess the reduction of hemolysis by eculizumab. Although LDH may be elevated due to other tissue damage or bone marrow failure associated with MDS etc., whether or not an elevated LDH is associated with hemolysis can be determined by checking for increased free Hb, decreased haptoglobin, and decreased PNH RBCs. In clinical studies, since treatment with eculizumab resulted in lowering of LDH levels with decreased free Hb, increased haptoglobin, and increased PNH RBCs, LDH isozyme analysis was not performed, but it was thought that hemolysis inhibition was related to lowering of LDH levels.

PMDA's view on the primary endpoints for the main clinical studies is as follows:

For PNH patients presenting with progressive anemia due to chronic intravascular hemolysis, hemolysis inhibition resulting in Hb stabilization and reduced transfusion requirements has clinical significance.

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Therefore, choosing "Hb stabilization" and "the number of PRBC units transfused" as the primary endpoints for the TRIUMPH study was appropriate for demonstrating the clinical significance of eculizumab.

On the other hand, although PMDA understands that the applicant used "LDH AUC" and "change of LDH" chosen as the primary endpoints for the SHEPHERD study and Study C07-001, respectively, as a measure of LDH reduction due to hemolysis inhibition, because LDH levels are known to change also in various diseases, isozyme analysis should have been performed. However, elevated LDH levels in PNH patients are considered due mainly to hemolysis and in light of the relationship to changes in free Hb, haptoglobin, and PNH RBCs etc., elevated LDH levels observed in clinical studies are considered due mainly to hemolysis. Thus, taking account of the mechanism of action of eculizumab, LDH can be used as a measure that indirectly assess hemolysis inhibition. Meanwhile, since clinically significant LDH AUC and LDH changes were not defined prior to their use as the endpoints, a problem still remains as to using these as a measure of treatment effect. The efficacy data from the SHEPHERD study and Study C07-001 demonstrated reduction of hemolysis by eculizumab rather than the favourable treatment effect of eculizumab in enrolled patients with PNH.

4.(iii).B.(4).2) Efficacy evaluation in the TRIUMPH study

For the co-primary endpoints in the TRIUMPH study, the Hb stabilization rate was 0.0% (0 of 44 patients) in the placebo group and 48.8% (21 of 43 patients) in the eculizumab group ($P < 0.001$, Fisher's exact test) and the number of PRBC units transfused (median [min., max.]) was 10 units [2 units, 21 units] in the placebo group and 0 units [0 units, 16 units] in the eculizumab group ($P < 0.001$, Wilcoxon's rank sum test). There were significant differences between the groups for both endpoints. Hb was maintained at levels that did not necessitate blood transfusion and transfusion requirements were reduced in more patients in the eculizumab group than in the placebo group among the PNH patients enrolled into the TRIUMPH study. Therefore, PMDA concluded that the clinical significance of hemolysis inhibition by eculizumab was demonstrated. However, as only 48.8% (21 of 43 patients) of patients achieved Hb stabilization, PMDA considered that there is a possibility that adequate treatment effect was not achieved in the study population as a whole.

4.(iii).B.(4).3) Comparison of the eligibility criteria among the main clinical studies

PMDA asked the applicant to compare the eligibility criteria among the TRIUMPH study, SHEPHERD study, and Study C07-001 and explain the reasons for differences in the criteria.

The applicant responded as follows:

The main common eligibility criteria among the 3 studies are shown in Table 20, the main different eligibility criteria are shown in Table 21, and the demographics and baseline characteristics of enrolled patients are shown in Table 22.

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Table 20. Main common eligibility criteria among the TRIUMPH study, SHEPHERD study, and Study C07-001

Inclusion criteria	PNH type III RBCs ≥ 10%, LDH ≥ 1.5 times the upper limit of laboratory reference range[a)]
Exclusion criterion	Neutrophil count < 500/μL

a) 103 to 223 U/L in the TRIUMPH and SHEPHERD studies; 120 to 240 U/L in Study C07-001

Table 21. Main different eligibility criteria among the TRIUMPH study, SHEPHERD study, and Study C07-001

	TRIUMPH	SHEPHERD	C07-001
Age	≥ 18 years	≥ 18 years	≥ 12 years
Transfusion	≥ 4 transfusions in the past 12 months for anemia or anemia-related symptoms	≥ 1 transfusion in the past 2 years for anemia or anemia-related symptoms or personal beliefs that precluded transfusions	≥ 1 transfusion in the past 2 years for anemia or anemia-related symptoms or personal beliefs that precluded transfusions
Platelet count	≥ 100 000/μL	≥ 30 000/μL	≥ 30 000/μL
Hb level	Pre-transfusion Hb ≤ 10.5 g/dL over the past 12 months	Not specified	Not specified

Table 22. Demographics and baseline characteristics of patients in the TRIUMPH study, SHEPHERD study, and Study C07-001

		Overseas			Japan
		TRIUMPH		SHEPHERD	C07-001
		Placebo	Eculizumab	N = 97	N = 29
		N = 44	N = 43		
Age (Mean ± SD)		38.45 ± 13.43 years	42.26 ± 15.45 years	41.11 ± 14.40 years	47.40 ± 12.39 years
Body weight (Mean ± SD)		72.8 ± 14.04 kg	74.94 ± 11.69 kg	73.72 ± 14.29 kg	61.48 ± 11.32 kg
LDH (Mean ± SD)		2258.0 ± 1027.1 U/L	2199.7 ± 1033.8 U/L	2199.8 ± 1034.4 U/L	1845.1 ± 621.14 U/L
Hb level (Mean ± SD)		9.63 ± 1.20 g/dL	10.01 ± 1.20 g/dL	9.19 ± 1.57 g/dL	7.90 ± 1.67 g/dL
PNH RBCs (Mean ± SD)		1.26 ± 0.69 × 10^{12}/L	1.12 ± 0.49 × 10^{12}/L	1.32 ± 0.75×10^{12}/L	1.26 ± 0.74 × 10^{12}/L
Percentage of PNH type III RBC clone (Mean ± SD)		35.14 ± 18.17%	27.98 ± 13.26%	38.72 ± 21.34%	43.89 ± 24.01%
Platelet count (Mean ± SD)		157.07 ± 76.67 × 10^9/L	188.84 ± 83.33 × 10^9/L	141.36 ± 78.54 × 10^9/L	146.2 ± 12.21 × 10^9/L
PNH duration (Mean ± SD)		10.7 ± 8.75 years	7.19 ± 7.21 years	7.41 ± 6.82 years	10.40 ± 6.14 years
History of AA or MDS (%)	AA	27.3% (N = 12)	14.0% (N = 6)	30.9% (N = 30)	37.9% (N = 11)
	MDS	0.0% (N = 0)	4.7% (N = 2)	1.0% (N = 1)	6.9% (N = 2)
No. of transfusions in the past 12 months (Mean ± SE)		9.0 ± 0.62	8.5 ± 0.61	5.9 ± 0.60	8.6 ± 1.50
No. of PRBC units transfused in the past 12 months (Mean ± SE)		19.8 ± 1.40 units	19.2 ± 1.28 units	12.2 ± 1.26 units	18.0 ± 3.02 units

It is difficult to directly compare the amount of PRBC transfused between Japan and overseas because the volume of 1 unit of PRBC is different between Japan and overseas and the volume of 1 unit of PRBC used at each trial site was not documented in clinical studies.

PMDA considers as follows:

The eligibility criteria for the SHEPHERD study and Study C07-001 were almost the same except for age. On the other hand, the eligibility criteria as to transfusion requirements prior to study entry, platelet count, and Hb level were different between these 2 studies and the TRIUMPH study. While only patients whose bone marrow was relatively spared and who were highly transfusion-dependent were eligible for the TRIUMPH study, patients with decreased bone marrow function and patients who required relatively fewer transfusions were also eligible for the SHEPHERD study and Study C07-001.

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With regard to the demographics and baseline characteristics of patients enrolled into these clinical studies, it is difficult to directly compare the amount of PRBC transfused because the specification for PRBC is different between Japan and overseas and the volume of 1 unit of PRBC used at each trial site was not documented, but patients in the SHEPHERD study and Study C07-001 tended to have lower platelet counts compared with patients in the TRIUMPH study. Patients in the C07-001 study tended to be older and have lower body weight, longer PNH duration, and lower LDH and Hb levels than patients in the TRIUMPH and SHEPHERD studies.

As described above, because differences in the demographics and baseline characteristics of patients among the studies were found, PMDA decided that subgroup analyses of the main clinical studies should be performed to identify the background factors affecting the efficacy of eculizumab [see "4.(iii).B.(4).5) Background factors affecting the efficacy of eculizumab"].

4.(iii).B.(4).4) Comparison of efficacy in the main clinical studies

PMDA asked the applicant to compare and present the results of the efficacy endpoints (Hb stabilization, the number of PRBC units transfused, LDH levels over time, change of LDH, LDH AUC) in the TRIUMPH study, SHEPHERD study, and Study C07-001.

The applicant explained as follows:

The number of PRBC units transfused, LDH levels over time, change of LDH, and LDH AUC up to Week 26 are presented in Table 23. "Hb stabilization" rates could not be calculated for the SHEPHERD study and Study C07-001 because the Hb concentration that necessitated transfusion during the observation phase (the set-point) had not been measured.

PMDA's view on comparison of efficacy in the studies is as follows:

Since "Hb stabilization" rates could not be calculated for the SHEPHERD study or Study C07-001, comparison with that in the TRIUMPH study is impossible. As previously noted, it is also difficult to simply compare "the number of PRBC units transfused" because the specification for PRBC is different between Japan and overseas and the volume of 1 unit of PRBC used at each trial site was not documented. Namely, the efficacy of eculizumab in the SHEPHERD study and Study C07-001 could not be compared with that in the TRIUMPH study in terms of improvement of anemia as indicated by "Hb stabilization" or transfusion requirements as indicated by "the number of PRBC units transfused." However, the SHEPHERD study and Study C07-001 also demonstrated a reduction in LDH from baseline, which is an indirect indicator of reduction in hemolysis, and a reduction in the number of PRBC units transfused from baseline.

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Table 23. Comparison of efficacy in the main clinical studies (TRIUMPH, SHEPHERD, C07-001)

		Overseas			Japan
		TRIUMPH		SHEPHERD	C07-001
		Placebo	Eculizumab		
		N = 44	N = 43	N = 97	N = 29
PRBC units transfused	12 weeks prior to start of treatment	4.5 [2.0, 15.0]	6.0 [2.0, 9.0]	2.0 [0.0, 12.0]	2.0 [0.0, 22.0]
	12 weeks after start of treatment	4.0 [0.0, 12.0]	0.0 [0.0, 8.0]	0.0 [0.0, 14.0]	0.0 [0.0, 14.0]
	26 weeks after start of treatment	10.0 [2.0, 21.0]	0.0 [0.0, 16.0]	0.0 [0.0, 26.0]	–
LDH	Baseline (U/L)	2234.5 [a), c)] [636.0, 5530.0]	2032.0 [a)] [499.0, 5962.0]	2051.0 [a)] [537.0, 5245.0]	1814.0 [b)] [627.8, 3642.5]
	Week 1 (U/L)	2089.0 [c)] [915.0, 4753.0]	621.0 [249.0, 2453.0]	580.0 [e)] [158.0, 3166.0]	542.0 [308.0, 2230.0]
	Week 12 (U/L)	2204.0 [d)] [550.0, 4682.0]	254.0 [d)] [136.0, 6067.0]	277.0 [f)] [120.0, 4147.0]	244.0 [187.0, 2715.0]
	Week 26 (U/L)	2166.5 [1183.0, 5643.0]	239.0 [d)] [142.0, 7692.0]	270.0 [g)] [98.0, 2944.0]	–
	Change at Week 12 (U/L)	145.0 [-1578.0, 3751.0]	-1739.0 [-4138.0, 105.0]	-1758.0 [f)] [-5008.0, 975.0]	-1327 [-3166.0, 436.3]
	Change at Week 26 (U/L)	57.0 [c)] [-1426.0, 1859.0]	-1840.0 [d)] [-4065.0, -300.0]	-1795.0 [g)] [-4678.0, 931.0]	–
	LDH AUC up to Week 12 (U/L·day)	4894.8 [-164 666.5, 138 509.0]	-142 387.0 [-333 776.5, -21 437.5]	-131 723.0 [-392 532.0, -20 126.0]	-107 613.6 [-238 162.8, 10 628.1]
	LDH AUC up to Week 26 (U/L·day)	–	-313 853.0 [-733 639.0, -50 767.5]	-301 837.0 [-881 797, -39 392.5]	–

Median [Min., Max.], –: Not calculable

a) Baseline LDH (Missing data were imputed from the last visit)

b) Baseline LDH was defined as mean LDH over the 4 weeks prior to start of treatment

c) N = 43

d) N = 41

e) N = 94

f) N = 95

g) N = 96

4.(iii).B.(4).5) Background factors affecting the efficacy of eculizumab

PMDA asked the applicant to explain the influences of patient background factors on the efficacy of eculizumab.

The applicant explained as follows:

Subgroup analyses by baseline levels of transfusion requirements and Hb, which are considered an indicator of hemolysis characteristic of PNH, and baseline level of platelet count, which is considered an indicator of bone marrow function, were performed to examine the influences of these patient background factors on the efficacy endpoints (Hb stabilization, the number of PRBC units transfused). The influences of age, PNH duration, and body weight, which tended to be different between Japanese and foreign clinical studies, were examined. Furthermore, in order to assess the effects of bone marrow function and the severity of PNH, the percentage of PNH type III RBCs and concurrent AA or MDS were examined.

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(a) Transfusion requirements

As shown in Table 24, regardless of transfusion requirements in the prior 12 months, the Hb stabilization rate was higher and the number of PRBC units transfused was fewer in the eculizumab group than in the placebo group in the TRIUMPH study.

In the TRIUMPH study, SHEPHERD study, and Study C07-001, when patients were stratified by transfusion requirement in the prior 12 months, there was no consistent trend in the number of PRBC units transfused by transfusion requirement in the prior 12 months.

Table 24. Influence of baseline transfusion requirements on efficacy

					Transfusion requirements in the prior 12 months (Unit)			Overall
					4-14	15-25	≥ 26	
Hb stabilization[a)]	Up to Week 12	TRIUMPH	Placebo	N = 44	6.7% (1/15)	5.6% (1/18)	0.0% (0/11)	4.5% (2/44)
			Eculizumab	N = 43	100.0% (15/15)	41.2% (7/17)	63.6% (7/11)	67.4% (29/43)
	Up to Week 26	TRIUMPH	Placebo	N = 44	0.0% (0/15)	0.0% (0/18)	0.0% (0/11)	0.0% (0/44)
			Eculizumab	N = 43	80.0% (12/15)	29.4% (5/17)	36.4% (4/11)	48.8% (21/43)
PRBC units transfused[b)]	Up to Week 12	TRIUMPH	Placebo	N = 44	2.0 [0.0, 6.0] (N = 15)	4.0 [0.0, 9.0] (N = 18)	8.0 [3.0, 12.0] (N = 11)	4.0 [0.0, 12.0] (N = 44)
			Eculizumab	N = 43	0.0 [0.0, 0.0] (N = 15)	0.0 [0.0, 6.0] (N = 17)	0.0 [0.0, 9.0] (N = 11)	0.0 [0.0, 9.0] (N = 43)
		SHEPHERD		N = 97	0.0 [0.0, 8.0] (N = 62)	0.5 [0.0, 8.0] (N = 12)	2.0 [0.0, 14.0] (N = 23)	0.0 [0.0, 14.0] (N = 97)
		C07-001		N = 29	0.5 [0.0, 3.0] (N = 15)	1.0 [0.0, 12.0] (N = 4)	0.5 [0.0, 13.0] (N = 10)	0.64 [0.0, 13.0] (N = 29)
	Up to Week 26	TRIUMPH	Placebo	N = 44	6.0 [2.0, 12.0] (N = 15)	10.0 [2.0, 12.0] (N = 18)	18.0 [10.0, 20.0] (N = 11)	10.0 [2.0, 21.0] (N = 44)
			Eculizumab	N = 43	0.0 [0.0, 4.0] (N = 15)	2.0 [0.0, 15.0] (N = 17)	3.0 [0.0, 16.0] (N = 11)	0.0 [0.0, 16.0] (N = 43)
		SHEPHERD		N = 97	0.0 [0.0, 16.0] (N = 62)	0.0 [0.0, 15.0] (N = 12)	3.0 [0.0, 26.0] (N = 23)	0.0 [0.0, 26.0] (N = 97)

[a)] Hb stabilization rate (N)
[b)] Median [Min., Max.] (N)

(b) Hb levels

As shown in Table 25, regardless of Hb levels prior to study entry, the Hb stabilization rate was higher and the number of PRBC units transfused was fewer in the eculizumab group than in the placebo group in the TRIUMPH study.

In the TRIUMPH study, SHEPHERD study, and Study C07-001, when patients were stratified by baseline Hb level, there was no consistent trend in the number of PRBC units transfused by baseline Hb level.

(c) Platelet count

As shown in Table 26, regardless of platelet counts prior to study entry, the number of PRBC units transfused was fewer in the eculizumab group than in the placebo group in the TRIUMPH study. On the other hand, although there was no difference in the Hb stabilization rate between placebo- and

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eculizumab-treated patients among the subgroup of patients with a baseline platelet count ≥ 50000/μL and < 100000/μL at Week 26, the Hb stabilization rate was higher in eculizumab-treated patients than in placebo-treated patients among the subgroups of patients with a baseline platelet count ≥ 100000/μL.

In the three studies, when patients were stratified by baseline platelet count, there was no consistent trend in the number of PRBC units transfused by baseline platelet count.

Table 25. Influence of baseline Hb levels on efficacy

					Hb levels prior to study entry (g/dL)				Overall
					< 7.0	≥ 7.0 to < 9.0	≥ 9.0 to < 11.0	≥ 11.0	
Hb stabilization[a)]	Up to Week 12	TRIUMPH	Placebo	N = 44	– (N = 0)	0.0% (0/13)	4.3% (1/23)	14.3% (1/7)	4.7% (2/43)
			Eculizumab	N = 43	100.0% (1/1)	50.0% (3/6)	66.7% (20/30)	83.3% (5/6)	67.4% (29/43)
	Up to Week 26	TRIUMPH	Placebo	N = 44	– (N = 0)	0.0% (0/13)	0.0% (0/23)	0.0% (0/7)	0.0% (0/43)
			Eculizumab	N = 43	100.0% (1/1)	33.3% (2/6)	43.3% (13/30)	83.3% (5/6)	48.8% (21/43)
PRBC units transfused[b)]	Up to Week 12	TRIUMPH	Placebo	N = 44	– (N = 0)	7.0 [3.0, 12.0] (N = 13)	4.0 [0.0, 9.0] (N = 23)	2.0 [0.0, 8.0] (N = 7)	4.0 [0.0, 12.0] (N = 43)
			Eculizumab	N = 43	0.0 [0.0, 0.0] (N = 1)	1.5 [0.0, 9.0] (N = 6)	0.0 [0.0, 8.0] (N = 30)	0.0 [0.0, 0.0] (N = 6)	0.0 [0.0, 9.0] (N = 43)
		SHEPHERD		N = 97	2.0 [0.0, 14.0] (N = 10)	0.0 [0.0, 9.0] (N = 33)	0.0 [0.0, 10.0] (N = 42)	0.0 [0.0, 8.0] (N = 12)	0.0 [0.0, 14.0] (N = 97)
		C07-001		N = 29	0.0 [0.0, 13.0] (N = 8)	0.0 [0.0, 12.0] (N = 15)	0.0 [0.0, 0.0] (N = 5)	0.0 [0.0, 0.0] (N = 1)	0.0 [0.0, 13.0] (N = 29)
	Up to Week 26	TRIUMPH	Placebo	N = 44	– (N = 0)	16.0 [5.0, 21.0] (N = 13)	8.0 [2.0, 20.0] (N = 23)	8.0 [6.0, 20.0] (N = 7)	10.0 [2.0, 21.0] (N = 44)
			Eculizumab	N = 43	0.0 [0.0, 0.0] (N = 1)	4.0 [0.0, 12.0] (N = 6)	1.0 [0.0, 16.0] (N = 30)	0.0 [0.0, 2.0] (N = 6)	0.0 [0.0, 16.0] (N = 43)
		SHEPHERD		N = 97	2.5 [0.0, 26.0] (N = 10)	2.0 [0.0, 19.0] (N = 33)	0.0 [0.0, 3.0] (N = 42)	0.0 [0.0, 15.0] (N = 12)	0.0 [0.0, 26.0] (N = 97)

a) Hb stabilization rate (N)
b) Median [Min., Max.] (N)

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Table 26. Influence of baseline platelet counts on efficacy

					Platelet counts prior to study entry (/μL)					Overall
					< 30 000	≥ 30 000 to < 50 000	≥ 50 000 to < 100 000	≥ 100 000 to < 150 000	≥ 150 000	
Hb stabilization[a)]	Up to Week 12	TRIUMPH	Placebo	N = 44	– (N = 0)	– (N = 0)	0.0% (0/7)	0.0% (0/16)	10.5% (2/19)	4.8% (2/42)
			Eculizumab	N = 43	– (N = 0)	– (N = 0)	75.0% (3/4)	46.2% (6/13)	76.9% (20/26)	67.4% (29/43)
	Up to Week 26	TRIUMPH	Placebo	N = 44	– (N = 0)	– (N = 0)	0.0% (0/7)	0.0% (0/16)	0.0% (0/19)	0.0% (0/42)
			Eculizumab	N = 43	– (N = 0)	– (N = 0)	0.0% (0/4)	38.5% (5/13)	61.5% (16/26)	48.8% (21/43)
PRBC units transfused[b)]	Up to Week 12	TRIUMPH	Placebo	N = 44	– (N = 0)	– (N = 0)	6.0 [2.0, 8.0] (N = 7)	4.5 [2.0, 12.0] (N = 16)	3.0 [0.0, 8.0] (N = 19)	4.0 [0.0, 12.0] (N = 42)
			Eculizumab	N = 43	– (N = 0)	– (N = 0)	0.0 [0.0, 6.0] (N = 4)	2.0 [0.0, 8.0] (N = 13)	0.0 [0.0, 6.0] (N = 26)	0.0 [0.0, 9.0] (N = 43)
		SHEPHERD		N = 97	4.5 [0.0, 9.0] (N = 2)	0.0 [0.0, 6.0] (N = 6)	2.0 [0.0, 14.0] (N = 27)	0.0 [0.0, 8.0] (N = 19)	0.0 [0.0, 8.0] (N = 43)	0.0 [0.0, 14.0] (N = 97)
		C07-001		N = 29	7.5 [2.0, 13.0] (N = 2)	0.0 [0.0, 0.0] (N = 1)	5.0 [0.0, 12.0] (N = 4)	0.0 [0.0, 0.0] (N = 8)	0.0 [0.0, 3.0] (N = 14)	0.0 [0.0, 13.0] (N = 29)
	Up to Week 26	TRIUMPH	Placebo	N = 44	– (N = 0)	– (N = 0)	15.0 [6.0, 20.0] (N = 7)	11.5 [5.0, 21.0] (N = 16)	8.0 [2.0, 18.0] (N = 19)	10.0 [2.0, 21.0] (N = 42)
			Eculizumab	N = 43	– (N = 0)	– (N = 0)	4.5 [2.0, 15.0] (N = 4)	6.0 [0.0, 16.0] (N = 13)	0.0 [0.0, 12.0] (N = 26)	0.0 [0.0, 16.0] (N = 43)
		SHEPHERD		N = 97	9.5 [0.0, 19.0] (N = 2)	3.5 [0.0, 14.0] (N = 6)	2.0 [0.0, 26.0] (N = 27)	0.0 [0.0, 15.0] (N = 19)	0.0 [0.0, 16.0] (N = 43)	0.0 [0.0, 26.0] (N = 97)

a) Hb stabilization rate (N)
b) Median [Min., Max.] (N)

(d) Age and body weight

With respect to age, younger patients tended to achieve Hb stabilization and require fewer PRBC units. PMDA is currently asking the applicant about the results on the influence of body weight.

(e) Percentage of PNH type III RBCs, PNH duration, and concurrent AA or MDS

There was no consistent trend for the influence of the percentage of PNH type III RBCs, PNH duration, or concurrent AA or MDS.

PMDA's view on the association between the patient background factors and efficacy is as follows: Regardless of the patient background factors, the number of PRBC units transfused was fewer in the eculizumab group than in the placebo group in the TRIUMPH study. The Hb stabilization rate tended to decrease with higher transfusion requirement in the 1 year prior to study treatment (Table 24), which indicated the possibility that adequate effect can not be achieved in patients with higher transfusion requirements.

Patients with low baseline Hb or platelet count tended to be unlikely to achieve Hb stabilization and

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transfusion avoidance (Table 25 and Table 26). There has so far been no information that there are differences in efficacy between PNH patients with and without AA or MDS.

Based on the above, although it was suggested that patients with higher baseline transfusion requirements and patients with low baseline Hb or platelet count tended to be unlikely to achieve Hb stabilization, it does not mean that treatment effect was not achieved, taking into account that the number of PRBC units transfused tended to decrease. Therefore, it is necessary to administer eculizumab to appropriate patients, taking account of clinical treatment effect and safety [see "4.(iii).B.(5) Safety"] needed for each patient, referring to the main study data.

Especially, in a patient population not included in the TRIUMPH study, reduction in hemolysis has been demonstrated, but treatment effect, e.g. Hb stabilization, has not been studied. Thus, information should continue to be collected via post-marketing surveillance etc. and should be provided to the clinical practice as appropriate.

PMDA is currently asking the applicant about the results on the influence of body weight.

4.(iii).B.(4).6) Long-term efficacy of eculizumab

LDH levels over time in the SHEPHERD study with a treatment duration of 52 weeks are shown in Table 27.

Table 27. LDH levels over time in the SHEPHERD study

Week	Week 0 (Baseline)	Week 1	Week 2	Week 4	Week 8	Week 12	Week 26	Week 52
N	97	94	97	96	95	95	96	94
LDH (U/L)	2051.0 [537.0, 5245.0]	580.0 [158.0, 3166.0]	351.0 [149.0, 1933.0]	262.5 [93.0, 1208.0]	284.0 [118.0, 3381.0]	277.0 [120.0, 4147.0]	270.0 [98.0, 2944.0]	269.0 [106.0, 2117.0]

Median [Min., Max.]

As shown in Table 27, lowering of LDH levels was sustained in the SHEPHERD study. Thus, PMDA considered that treatment with eculizumab results in sustained decrease in hemolysis.

PMDA is currently asking the applicant about LDH levels over time and transfusion requirements over time based on pooled analyses of the submitted foreign clinical studies (TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, X03-001A, E05-001).

4.(iii).B.(4).7) Lack of efficacy of eculizumab

Since treatment discontinuation due to lack of efficacy occurred in Study C07-001 only (2 patients) among the main clinical studies (TRIUMPH, SHEPHERD, C07-001), PMDA asked the applicant to explain its cause.

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The applicant responded as follows:
In Study C07-001, 2 patients did not show a pharmacodynamic response as measured by LDH levels. In an *in vitro* assay using serum samples from these 2 patients, serum complement activity was not inhibited by eculizumab, but hemolytic activity was inhibited by an anti-C5 antibody that is slightly different in structure from eculizumab (ALXN-Ab). Therefore, there is a possibility that eculizumab did not bind to C5 due to a functional variation in C5, but the cause is undefined and a detailed investigation of C5 in serum from these patients will be conducted.

PMDA considers as follows:
As lack of efficacy was observed in the Japanese clinical study only, the possibility that there are differences in efficacy between Japan and overseas also can not be ruled out, but the cause is undefined and it is necessary to continue to collect information on ineffective cases via post-marketing surveillance etc. and provide the information to the clinical practice as appropriate. Except for these 2 patients, as shown in Tables 23 and 27, LDH levels were reduced by Week 1. Therefore, if LDH levels are not reduced by Week 1, whether or not to continue treatment should be considered.

As lack of efficacy has been reported from foreign post-marketing surveillance (Table 32), PMDA is currently asking the applicant about the details.

4.(iii).B.(4).8) Efficacy against TE
TE is one of the leading causes of death in PNH and thrombus formation is considered attributed to platelet activation induced by increased free Hb concentration due to intravascular hemolysis. According to a foreign single-center, retrospective study with a 6-year [0.2 years, 38 years] (median [min., max.]) follow-up of 163 PNH patients, there were 19 TE events in 511.5 patient-years in 67 patients without history of TE and not taking warfarin; and the incidence of TE in PNH was estimated to be 3.7 per 100 patient-years (*Blood*. 2003;102:3587-3591.). On the other hand, the incidence of TE in the general population was 5.04 per 10000 person-years (*Eur J Vasc Endovasc Surg*. 2003;25:1-5.). A higher incidence of TE in PNH patients has been suggested.

With regard to the effect of eculizumab on TE, 1 TE event occurred in the placebo group and 0 TE event occurred in the eculizumab group in the TRIUMPH study. The incidence of TE was 2.34 per 100 patient-years prior to the start of treatment (calculated from previous TE events in the past) and 4.38 per 100 patient-years after the start of treatment in the placebo group, compared with 5.18 per 100 patient-years prior to the start of treatment and 0.00 per 100 patient-years after the start of treatment in the eculizumab group. Based on the pooled data from eculizumab-treated patients in foreign clinical studies (TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, E05-001), the incidence of TE was 7.37 per 100 patient-years before the start of eculizumab treatment compared with 1.07 per 100 patient-years after eculizumab treatment.

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On the other hand, in the Japanese study C07-001, 5 of 29 patients had previously experienced 5 TE events and the incidence of TE was 1.66 per 100 patient-years before the start of treatment, but TE did not occur in the 3-month study period.

PMDA considers as follows:
The above results were not based on studies intended to investigate the incidence of TE and the detailed background information on concurrent use of anticoagulants, etc. is unknown and the possible influences of other factors also can not be excluded. However, foreign clinical studies have suggested that treatment with eculizumab may reduce the incidence of TE. The prevention of TE in PNH patients has a significant impact on survival prognosis. On the other hand, as it has been reported that the mortality from TE as well as the incidence of TE are lower in Japanese PNH patients than in foreign PNH patients [*Medicine*. 2004;83:193-207, see "4.(iii).B.(2) Differences in PNH between Japan and overseas"], the clinical benefits obtained from the prevention of TE may be different between Japanese PNH patients and PNH patients in the US/Europe. Therefore, the information on the development of TE, concurrent use of anticoagulants and other TE risk factors, etc. should be collected via post-marketing surveillance, differences between Japan and overseas should also be investigated, and then the information should be provided to the medical practice as appropriate.

4.(iii).B.(4).9) Efficacy during an acute hemolytic crisis
Since PNH patients are known to develop an acute hemolytic crisis associated with infections or trauma, PMDA asked the applicant to explain if there is clinical experience with eculizumab during an acute hemolytic crisis and whether or not to use eculizumab during an acute hemolytic crisis.

The applicant responded as follows:
Although there were no patients who started treatment with eculizumab after the onset of an acute hemolytic crisis associated with infections or trauma in clinical studies in PNH patients, the case of a PNH patient who developed an acute hemolytic crisis after delivery and started treatment with eculizumab has been reported from the foreign marketing experience. The patient received dialysis due to acute renal failure following the acute hemolytic crisis, but LDH levels returned to normal following treatment with eculizumab and the renal function was normalized; and dialysis was discontinued. Then, the patient was discharged and received treatment with eculizumab on an outpatient basis and was lost to follow-up thereafter.

On the other hand, the cases of hemolysis associated with infections or trauma during treatment with eculizumab (breakthrough hemolysis) were investigated. In the TRIUMPH and SHEPHERD studies, a total of 2 PNH patients experienced severe hemolysis associated with trauma during treatment with eculizumab, but continued treatment. Therefore, even if a patient develops an acute hemolytic crisis during treatment with eculizumab, treatment may be continued carefully at the discretion of the

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physician.

PMDA considers as follows:
Theoretically, eculizumab is expected to be effective during an acute hemolytic crisis, as with in the setting of chronic hemolysis, but there is very limited clinical experience and the efficacy and safety information is insufficient. Thus, it is difficult to recommend the use of eculizumab for treating acute hemolytic crises. However, if patients treated with eculizumab during an acute hemolytic crisis are identified after the market launch, it is important to collect information and provide information on actions to be taken in the event of an acute hemolytic crisis to the clinical practice as appropriate.

4.(iii).B.(5) Safety
As a result of the following safety reviews on comparison of the eculizumab and placebo groups in the TRIUMPH study and on long-term safety of eculizumab etc., PMDA confirmed based on the clinical study data that special attention should be paid to the possible occurrence of headache. Furthermore, as it is inferred that patients treated with eculizumab are at an increased risk for infections with encapsulated bacteria including *Neisseria meningitidis*, PMDA reviewed the issue of the risk of meningococcal infection, including its incidence in the foreign marketing experience [see "4.(iii).B.(5).4) Meningococcal infections"]. Especially, as measures against *Neisseria meningitidis* are an important issue for the safety of eculizumab, eculizumab should be used after carefully balancing the clinical benefits and the risks. The package insert should contain stringent warnings and precautions and a fully prepared safety control system is needed.

A final conclusion on the safety of eculizumab will be made, taking account of comments from the Expert Discussion.

4.(iii).B.(5).1) Safety in the eculizumab group in the TRIUMPH study
In the TRIUMPH study, adverse events reported at a ≥ 5.0% higher incidence in the eculizumab group than in the placebo group were headache, nasopharyngitis, back pain, and fatigue (Table 15). There were no particular differences in serious adverse events.

The applicant discussed that inhibition of intravascular hemolysis by eculizumab leads to reduced free Hb, which scavenges nitric oxide (NO) and increased free NO dilates blood vessels, causing headache. PMDA is currently asking the applicant to discuss nasopharyngitis, back pain, and fatigue.

PMDA considers as follows:
No serious adverse events unique to the eculizumab group were reported and although the incidences of adverse events of headache, nasopharyngitis, back pain, and fatigue tended to be higher in the eculizumab group compared with the placebo group, headache occurred as a severe event in 1 patient

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each in the placebo and eculizumab groups and there were no other severe events. Thus, although attention should be paid to the possible occurrence of headache, the results of the placebo-controlled TRIUMPH study indicate that adverse events associated with eculizumab are tolerable.

4.(iii).B.(5).2) Long-term safety of eculizumab and the influences of patient background factors on safety

Adverse events occurring in ≥ 10.0% of patients in either group up to Week 12 in the TRIUMPH study, SHEPHERD study, and Study C07-001 are shown in Table 28. Adverse events occurring in ≥ 10.0% of patients in either group during the entire treatment period in pooled foreign clinical studies (TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, E05-001) are shown in Table 29. Serious adverse events occurring in at least 2 patients up to Week 12 were anemia (3.1% [3 of 97 patients]) in the SHEPHERD study only. Serious adverse events occurring in at least 2 patients during the entire treatment period in pooled foreign clinical studies (TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, E05-001) are shown in Table 30. Of the serious adverse events listed in Table 30, those for which a causal relationship to study drug could not be denied were pyrexia (2.6% [5 of 195 patients]), headache (1.5% [3 of 195 patients]), viral infection (1.0% [2 of 195 patients]), and septic shock (1.0% [2 of 195 patients]).

Table 28. Adverse events occurring in ≥ 10.0% of patients in either group up to Week 12

	TRIUMPH				SHEPHERD		C07-001	
	Placebo (N = 44)		Eculizumab (N = 43)		Eculizumab (N = 97)		Eculizumab (N = 29)	
	Incidence	N	Incidence	N	Incidence	N	Incidence	N
Overall	84.1%	37	93.0%	40	85.6%	83	96.6%	28
Headache	20.5%	9	39.5%	17	45.4%	44	51.7%	15
Nasopharyngitis	11.4%	5	18.6%	8	11.3%	11	41.4%	12
Nausea	6.8%	3	14.0%	6	11.3%	11	20.7%	6
Diarrhoea	6.8%	3	4.7%	2	6.2%	6	13.8%	4
Vomiting	6.8%	3	4.7%	2	7.2%	7	10.3%	3
Pyrexia	4.5%	2	2.3%	1	5.2%	5	10.3%	3
Eczema	0.0%	0	0.0%	0	0.0%	0	10.3%	3
Dizziness	11.4%	5	2.3%	1	6.2%	6	3.4%	1

Table 29. Adverse events occurring in ≥ 10.0% of patients in either group during the entire treatment period in foreign clinical studies (Pooled analysis)

	Placebo (N = 44)		Eculizumab (N = 195)			Placebo (N = 44)		Eculizumab (N = 195)	
	Incidence	N	Incidence	N		Incidence	N	Incidence	N
Overall	90.9%	40	99.0%	193	Dizziness	11.4%	5	15.4%	30
Headache	27.3%	12	51.3%	100	Pharyngolaryngeal pain	9.1%	4	14.4%	28
Nasopharyngitis	18.2%	8	42.1%	82	Pain in extremity	2.3%	1	14.4%	28
Upper respiratory tract infection	22.7%	10	30.8%	60	Abdominal pain	11.4%	5	12.8%	25
Nausea	11.4%	5	25.1%	49	Insomnia	6.8%	3	11.8%	23
Diarrhoea	11.4%	5	22.1%	43	Constipation	4.5%	2	11.8%	23
Back pain	9.1%	4	20.0%	39	Viral infection	11.4%	5	11.3%	22
Pyrexia	4.5%	2	16.9%	33	Urinary tract infection	9.1%	4	11.3%	22
Vomiting	11.4%	5	16.4%	32	Contusion	6.8%	3	11.3%	22
Arthralgia	9.1%	4	15.9%	31	Influenza like illness	2.3%	1	11.3%	22
Cough	9.1%	4	15.9%	31	Myalgia	2.3%	1	10.8%	21

Foreign studies: TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, and E05-001

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Table 30. Serious adverse events occurring in at least 2 patients in either group during the entire treatment period in foreign clinical studies (Pooled analysis)

	Placebo (N = 44)		Eculizumab (N = 195)			Placebo (N = 44)		Eculizumab (N = 195)	
	Incidence	N	Incidence	N		Incidence	N	Incidence	N
Overall	20.5%	9	25.6%	50	Thrombocytopenia	0.0%	0	1.0%	2
Pyrexia	2.3%	1	3.6%	7	Abdominal pain	0.0%	0	1.0%	2
Viral infection	2.3%	1	2.6%	5	Cholelithiasis	0.0%	0	1.0%	2
Anaemia	2.3%	1	2.1%	4	Viral gastroenteritis	0.0%	0	1.0%	2
Headache	0.0%	0	2.1%	4	Septic shock	0.0%	0	1.0%	2
Intervertebral disc protrusion	0.0%	0	1.5%	3	Convulsion	0.0%	0	1.0%	2
PNH	6.8%	3	1.0%	2	Nephrolithiasis	0.0%	0	1.0%	2
Cellulitis	2.3%	1	1.0%	2	Acute renal failure	0.0%	0	1.0%	2
Haemolytic anaemia	0.0%	0	1.0%	2	Neutropenia	4.5%	2	0.5%	1

Foreign studies: TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, and E05-001

Since eculizumab is an anti-C5 antibody and was considered to possibly affect the immune function, the incidence of malignant tumors was investigated.

According to foreign post-marketing surveillance (March 16, 2007 to April 1, 2009), 19 cases of neoplasms benign, malignant and unspecified (incl cysts and polyps) (acute myeloid leukaemia [3], MDS [3], skin cancer [2], acute leukaemia [1], basal cell carcinoma [1], brain neoplasm [1], endometrial cancer stage I [1], hepatic neoplasm malignant [1], leukaemia [1], lung adenocarcinoma [1], lymphoma [1], pseudolymphoma [1], renal cancer [1], uterine leiomyoma [1]) were reported, but their relationship to eculizumab is undefined at present.

On the other hand, PMDA asked the applicant to explain differences in safety profile according to patient background, based on the results from the TRIUMPH study, SHEPHERD study, and Study C07-001.

The applicant responded as follows:
Although the incidences of adverse events of nasopharyngitis, headache, and eczema etc. tended to be higher in Study C07-001 than in foreign studies, these differences are considered of no clinical significance because the diagnostic criteria are different between Japan and overseas.

The influences of body weight, age, the percentage of PNH type III RBCs, and reticulocyte count at baseline as the background factors were examined. As a result, there was no clinically significant consistent trend in the incidence of adverse events.

On the other hand, with respect to gender, the incidences of adverse events of headache, nausea, back pain, and abdominal pain tended to be higher in female patients than in male patients, but its cause is unknown. Although the incidence of headache tended to be higher in patients with fewer PRBC units transfused over the 1 year prior to the start of treatment, patients with lower baseline Hb, patients with

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lower baseline platelet counts, and patients with AA or MDS, the results were inconsistent across the studies.

PMDA's view on adverse events associated with eculizumab is as follows:
The safety data up to Week 12 from the Japanese and foreign clinical studies in PNH patients showed that the incidence of headache was higher in the eculizumab group than in the placebo group and most of the events occurred by Week 5 (the incidence of headache in eculizumab-treated patients in the main clinical studies [TRIUMPH, SHEPHERD, C07-001] was 34.7% [68 of 169 patients] up to Week 5 and 7.7% [13 of 169 subjects] in Weeks 6-12). The events of headache were mostly mild or moderate in severity, but during the entire treatment period including the period beyond 12 weeks (C02-001, E02-001, X03-001, TRIUMPH, SHEPHERD, E05-001, C07-001), severe headache occurred at an incidence of 4.9% (11 of 224 patients) and serious headache occurred at an incidence of 1.8% (4 of 224 patients). Thus, attention should be paid to the possible occurrence of headache, especially during the early phase of treatment.

Other than headache, only pyrexia occurred at an incidence of ≥ 10.0% and was reported as a severe adverse event in at least 2 patients during the entire treatment period including the period beyond 12 weeks and serious pyrexia also occurred at an incidence of 3.6% (8 of 224 patients). Thus, like headache, pyrexia deserves attention. There were no other particular adverse events that deserve clinical attention. There have so far been no events that increased in incidence or severity with prolonged treatment with eculizumab. However, since there is limited clinical experience with long-term treatment with eculizumab and eculizumab possibly affects the immune function, it is important to collect information on events occurring during long-term treatment, such as influences of eculizumab on occurrence of malignant tumors and leukemia, after the market launch and provide the information to the clinical practice as appropriate.

On the other hand, as it is difficult to compare in details the safety of eculizumab, including the influences of patient background factors, between Japan and overseas due to the limited number of patients in clinical studies, the possibility of differences in safety profile between Japan and overseas can not be ruled out, but the submitted study data do not suggest the presence of differences that would significantly affect the tolerability of eculizumab.

Although no background factor that limits the use of eculizumab or requires careful administration has been identified, it is necessary to continue to collect information and provide the information as appropriate.

The safety data (adverse events and serious adverse events) from pooled Japanese (C07-001, E07-001) and foreign (TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, X03-001A, E05-001) clinical

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studies are currently being requested.

4.(iii).B.(5).3) Foreign post-marketing data

Adverse events and serious adverse events reported in clinical studies in PNH patients (C07-001, E07-001, TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, X03-001A, E05-001) were compared with those reported from foreign post-marketing surveillance (from March 17, 2007 to April 1, 2009). The cumulative number of patients exposed to eculizumab was 281.03 patient-years in the PNH clinical studies and 1249 patient-years in the foreign post-marketing surveillance. Adverse events occurring at an incidence of ≥ 5.0 per 100 patient-years in either the clinical studies or foreign post-marketing surveillance are shown in Table 31 and serious adverse events occurring at an incidence of ≥ 1.0 per 100 patient-years in either the clinical studies or foreign post-marketing surveillance are shown in Table 32. No new safety issues were identified.

Table 31. Adverse events occurring at an incidence of ≥ 5.0 per 100 patient-years in either clinical studies or foreign post-marketing surveillance

Adverse event	Clinical studies		Foreign post-marketing surveillance		Adverse event	Clinical studies		Foreign post-marketing surveillance	
	Events per 100 patient-years	No. of events	Events per 100 patient-years	No. of events		Events per 100 patient-years	No. of events	Events per 100 patient-years	No. of events
Headache	40.0	115	19.5	244	Constipation	8.0	23	0.2	3
Nasopharyngitis	32.7	94	4.5	56	Urinary tract infection	7.6	22	1.7	21
Upper respiratory tract infection	20.8	60	0.7	9	Myalgia	7.6	22	1.0	13
Nausea	19.1	55	5.8	73	Flu-like symptoms	7.6	22	0.6	8
Diarrhoea	16.3	47	2.7	34	Contusion	7.6	22	0.6	8
Back pain	13.6	39	4.8	60	Viral infection	7.6	22	0.5	6
Pyrexia	12.5	36	6.2	77	Fatigue	7.3	21	6.6	83
Vomiting	12.2	35	3.0	37	Epistaxis	6.6	19	0.7	9
Arthralgia	11.1	32	2.6	32	Abdominal pain upper	5.6	16	1.2	15
Cough	11.1	32	1.4	18	Herpes labialis	5.6	16	0.4	5
Dizziness	10.8	31	2.6	33	Itching	5.2	15	1.2	15
Pain in extremity	10.1	29	1.8	23	Influenza	5.2	15	1.0	12
Pharyngolaryngeal pain	9.7	28	0.0	0	Hb decreased	0.0	0	9.7	121
Abdominal pain	8.7	25	3.4	42					

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Table 32. Serious adverse events occurring at an incidence of ≥ 1.0 per 100 patient-years in either clinical studies or foreign post-marketing surveillance

Serious adverse event	Clinical studies		Foreign post-marketing surveillance		Serious adverse event	Clinical studies		Foreign post-marketing surveillance	
	Events per 100 patient-years	No. of events	Events per 100 patient-years	No. of events		Events per 100 patient-years	No. of events	Events per 100 patient-years	No. of events
Pyrexia	2.8	8	4.0	50	Pneumonia	0.3	1	1.0	13
Viral infection	1.7	5	0.4	5	Pain	0.3	1	1.0	12
Headache	1.4	4	3.0	38	Hb decreased	0.0	0	4.6	57
Anaemia	1.4	4	2.2	28	Fatigue	0.0	0	2.2	27
Abdominal pain	0.7	2	1.7	21	Blood LDH increased	0.0	0	1.4	17
Dyspnoea	0.3	1	1.8	22	Asthenia	0.0	0	1.3	16
Back pain	0.3	1	1.5	19	Death	0.0	0	1.2	15
Sepsis	0.3	1	1.1	14	Lack of efficacy	0.0	0	1.1	14
Urinary tract infection	0.3	1	1.1	14	Platelet count decreased	0.0	0	1.1	14

Although PMDA is currently asking the applicant for its view on the safety of eculizumab based on the data from the post-marketing surveillance, PMDA found no particular events that were reported at a markedly higher incidence in the foreign post-marketing surveillance than in the clinical studies. However, since cases of lack of efficacy have been reported overseas, the details of these cases are currently being requested.

4.(iii).B.(5).4) Meningococcal infections

(a) The risk of developing a meningococcal infection in patients treated with eculizumab

Eculizumab blocks the formation of the terminal complement complex (C5b-9). As *Neisseria meningitidis* having a polysaccharide capsule is lysed by the terminal complement complex (C5b-9), as with patients with a genetic disease, late complement component deficiency (LCCD), patients treated with eculizumab are considered susceptible to infections with *Neisseria meningitidis*. Although the risk of developing a meningococcal infection in LCCD patients differs depending on which terminal complement component (C5b-9) is deficient, it has been reported that the risk of meningococcal disease among LCCD individuals is 1400- to 10000-fold greater than that in normal individuals (*Clin Microbiol.* 1991;Rev 4:359-395, *J Pediatr.* 1989;114:260-264.). Thus, patients treated with eculizumab are also expected to have a similar level of risk.

On the other hand, the clinical development of eculizumab for non-PNH patients was initiated in 19[redacted] and a total of 716 patients unvaccinated against *Neisseria meningitidis* received eculizumab. In Study E99-044 in patients with idiopathic membranous glomerulonephritis, 1 patient developed meningitis caused by *Neisseria meningitidis* infection. Considering the potential risk of infection based on the mechanism of action of eculizumab, Alexion (the US) decided to require patients to be immunized with

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a meningococcal vaccine prior to receiving eculizumab in subsequent clinical studies. The package inserts for eculizumab in the US/Europe state as follows: "Immunize patients with a meningococcal vaccine at least 2 weeks prior to administering the first dose of eculizumab and then revaccinate patients periodically." and "Eculizumab is contraindicated in patients who are not currently vaccinated with a meningococcal vaccine." However, a reduction in the risk of developing a meningococcal infection in eculizumab-treated patients immunized with a meningococcal vaccine has not been confirmed.

PMDA confirmed the following points:
· Based on its mechanism of action, eculizumab is expected to increase the risk of developing a meningococcal infection.
· The reason for requiring patients to be immunized with a meningococcal vaccine in clinical studies
· In the US/Europe where eculizumab has already been approved, the package insert requires meningococcal vaccination prior to administering eculizumab.

On the other hand, taking into account that a vaccine against *Neisseria meningitidis* is unapproved in Japan, etc., the applicant explained safety measures against meningococcal infections associated with the use of eculizumab in Japan as follows: meningococcal vaccination will not be required; the measures to reduce the risks associated with meningococcal infections, which are being implemented in foreign countries, will be employed; and a system for detecting meningococcal infections early and taking appropriate and prompt actions will be established.

Taking meningococcal infections and its safety measures seriously for the safety of eculizumab, PMDA reviewed the epidemiology of meningococcal infections, the status of meningococcal infections among patients treated with eculizumab, the effectiveness of meningococcal vaccines, and measures against meningococcal infections, as shown in (b) to (e).

(b) Epidemiology of meningococcal infections

i) Overview of meningococcal infections

The applicant explained the risk factors, clinical findings, and treatments of meningococcal infections as follows:
LCCD is known as a definitive risk factor for meningococcal infections and other risk factors include the carriage of *Neisseria meningitidis*, children at ≤ 4 years of age, and people in communal living settings at high risk of contagion.

Clinical findings of meningococcal infection include pyrexia, headache, photophobia, nuchal rigidity, mental status changes, convulsion, nausea, vomiting, and purpura/petechiae and usually, these symptoms occur acutely. Purpura/petechiae deserves attention because systemic manifestations of meningitis associated with high fatality rates, e.g. fulminant meningococcal sepsis accompanied by

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hypotension, shock, and multi-organ failure, occur.

Regarding treatments, although a definitive diagnosis of meningococcal infection is made with blood/cerebrospinal fluid culture, as meningococcal infection can progress rapidly, it is important to start administering a third-generation cephem antibiotic soon after the onset of symptoms ahead of test results, if meningococcal infection is suspected. Although *Neisseria meningitidis* is generally highly susceptible to antibiotics, the fatality rate for meningococcal meningitis is as high as 10% (*IASR*. 2005;26:33-34.), and if patients respond to treatment, 10% to 15% of survivors will be left with serious sequelae.

With regard to the outcomes of meningococcal infections according to serogroup, it has been reported that the fatality rate by serogroup was 7.3% (3 of 41 patients) for serogroup B, 10.5% (2 of 19 patients) for serogroup C, and 0.0% (0 of 6 patients) for serogroup W135 in Israel (*Infection*. 1999;27:261-264.) and 11% for serogroup B, 12% for serogroup C, 17% for serogroup Y, and 8% for serogroup W135 in Sweden (*Scand J Infect Dis*. 2008;40:734-744.). No trend towards marked differences in the fatality rate according to serogroup was observed and adequate attention should be paid to all serogroups.

The extent of the risk of infection with *Neisseria meningitidis* and the incidence and prognosis of meningococcal infection, etc., in patients treated with eculizumab are undefined.

ii) Differences in meningococcal infections between Japan and overseas

The applicant explained differences in the meningococcal carriage rate, the incidence of meningococcal disease, and meningococcal serogroups, etc. between Japan and overseas as follows:

The meningococcal carriage rate among healthy adults has been estimated to be 0.64% in Japan, which has been reported to be lower than those in foreign countries (16.7% in the UK, 9.6% in Norway, 16% in Israel) (*J Med Microbiol*. 2004;53:657-662, *Lancet*. 2002;359:1829-1831, *Epidemiol Infect*. 1999; 122:51-57, *J Clin Microbiol*. 1994; 32: 323-330.).

In Japan, more than 4000 cases of meningococcal infections were reported in around 1945, which was reduced to less than 100 cases per year after 1969 and then to less than 10 cases per year in 1990s. After the enforcement of the Infectious Diseases Control Law in 1999, 8 to 22 cases of meningococcal infections occurred annually. According to a survey conducted from April 1999 to December 2004, there were 82 cases of meningococcal infections, including 8 fatal cases (*IASR*. 2005;26:33-34.), indicating that the fatality rate is about 10%. On the other hand, according to an US epidemiological surveillance, the annual incidence decreased from 1.3/100,000 population in 1989 to 0.9/100,000 population in 1991 and increased from 0.8/100,000 population in 1992 to 1.0/100,000 population in 1996 and then the annual incidence was 0.8/100,000 population in 1997 and 0.9/100,000 population in 1998 (*J Infect Dis*. 1999;180:1894-1901.), and it has been suggested that the case-fatality rate is

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approximately 10% (*Emerg Infect Dis*. 2003;9:355-361.).

With respect to meningococcal serogroups, the serogroups of isolates for 182 cases collected between 1974 and 2003 in Japan were reported to be B (56.5%), Y (21.4%), W135 (0.5%), and unknown (21.4%) (*IASR*. 2005;26:36-37.) and the serogroups of isolates for 82 cases collected between April 1999 and December 2004 were reported to be B (26.8%), Y (18.3%), A (3.7%), C (1.2%), and unknown (50.0%) (*IASR* 26: 33-34, 2005). The serogroups of isolates for 807 cases collected between 1992 and 1996 in the US were C (35%), B (32%), and Y (26%) and the proportion of meningococcal cases due to serogroup Y tended to increase from 10.6% in 1992 to 32.6% in 1996 (*J Infect Dis*. 1999;180: 1894-1901.).

As shown in the above, the carriage rate, incidence, and serogroups differed from region to region and from period to period.

The information on the carriage of *Neisseria meningitidis* was not collected in clinical studies of eculizumab in PNH patients. Also in the foreign marketing experience, patients have not been tested for meningococcal carriage prior to receiving eculizumab and the information on the carriage of *Neisseria meningitidis* is not available.

There is no report suggesting that the carriage of *Neisseria meningitidis* in PNH patients is different from that in the general population.

Based on i) and ii), PMDA considers as follows:
Although it is understood that based on the presented reports, the meningococcal carriage rate is lower and the incidence of meningococcal disease is expected to be lower in the general population of Japan compared with foreign countries, as *Neisseria meningitidis* is transmitted easily by droplets from the upper respiratory tract of carriers, the risk of developing a meningococcal infection in patients treated with eculizumab is an important issue also in Japan where the carriage rate is low. Since the clinical course of meningococcal infection is generally rapid with a high fatality rate of around 10% and the rate of sequelae is also high, adequate caution and measures against meningococcal infections are needed.

(c) The occurrence and course of meningococcal infections in clinical studies and foreign marketing experience

The applicant explained the occurrence of meningococcal infections in clinical studies and marketing experience of eculizumab as follows:
Among a total of 911 patients treated with eculizumab in clinical studies up to [redacted], 3 patients developed a meningococcal infection. The cumulative number of patients exposed to eculizumab from the market launch to [redacted] overseas was estimated to be 1030 patient-years and 6 cases of

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meningococcal infections were reported (Periodic safety update report from [redacted], 20[redacted] to [redacted], 20[redacted]). Meningococcal infections reported in patients treated with eculizumab are outlined in Table 33.

PMDA considers as follows:
Among the patients who developed a meningococcal infection, all patients except for 1 patient with idiopathic membranous glomerulonephritis had been vaccinated with meningococcal vaccine. The timing of vaccination was unknown for the 2 cases in clinical studies. Among the 6 cases reported from the marketing experience, 4 cases were caused by serogroup B, which was not covered by the vaccine, and the remaining 2 cases were caused by serogroup Y or C in spite of vaccination with a tetravalent (serogroups A, C, Y, and W135) vaccine, indicating that vaccination can not completely protect against the development of meningococcal infection. However, in the cases of a 2[redacted]-year-old woman in the US with serogroup Y meningococcal disease and a 3[redacted]-year-old man in the UK with serogroup C meningococcal disease, the disease developed shortly (i.e. 1 month) after revaccination 3 years after the first vaccination and 27 months after vaccination, respectively. Therefore, it can not clearly be concluded that vaccination is ineffective.

The protective efficacy of vaccine against meningococcal infections in patients treated with eculizumab is not adequately proven because comparison with unvaccinated patients has not been made. In the clinical studies and foreign marketing experience, patients who developed a meningococcal infection in spite of vaccination with meningococcal vaccine were reported and it was confirmed that even vaccinated patients can develop a meningococcal infection. Although most of the patients with meningococcal infection recovered following antibiotic treatment, there were patients who died or were left with sequelae and it was confirmed that the development of meningococcal infection can lead to a serious outcome.

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Table 33. Patients of meningococcal infections reported in clinical studies and marketing experience

	Patient background (primary disease, country, sex, age)	Vaccine serogroups	Serogroup of infecting meningococcus	Time to onset		Event	Initial treatment (Antibiotics)	Outcome	Eculizumab was continued or not / Revaccination
				after vaccination	after eculizumab initiation				
Clinical studies	Idiopathic membranous glomerulonephritis US Female 2[redacted] years	Unvaccinated	A	—	—	Meningitis	Ceftriaxone Vancomycin Ampicillin	Recovered (with sequelae)	Unknown Unknown
	PNH US Male 2[redacted] years	A, C, Y, W135	B	Unknown	11 months	Sepsis	Ceftriaxone Vancomycin	Recovered	Unknown Unknown
	PNH Spain Female 5[redacted] years	A, C	B or Y or W135	Unknown	13 months	Sepsis	Ceftriaxone	Recovered	Unknown Unknown
Marketing experience	PNH US Female 4[redacted] years	A, C, Y, W135	B	6 months	4 months	Meningitis	Ceftriaxone	Recovered	Continued Unknown
	PNH Ireland Male 2[redacted] years	C	B	6 months	5 months	Sepsis	Cefotaxime Benzylpenicillin Gentamicin Metronidazole	Death	– –
	PNH US Female 2[redacted] years	A, C, Y, W135	Y	3 years after the first vaccination, 1 month after revaccination	2 months	Sepsis	Ceftriaxone	Recovered	Continued Unknown
	PNH UK Male 2[redacted] years	A, C, Y, W135	B	3 months	2 months	Infection	Ceftazidime	Recovered	Continued Unknown
	PNH Denmark Male 2[redacted] years	C	B	12 months	9 months (36 days after eculizumab discontinuation)	Sepsis	Ceftazidime	Recovered	Discontinued Unknown
	PNH UK Male 3[redacted] years	A, C, Y, W135	C	27 months after the first vaccination	21 months	Sepsis	Ceftriaxone	Recovered	Continued Revaccinated

(d) Effectiveness of meningococcal vaccines

The protective efficacy of tetravalent meningococcal vaccines, which are currently used overseas, as described in the product package inserts, are summarized in Table 34.

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Table 34. Efficacy rates of tetravalent meningococcal vaccines approved overseas

Brand name	Age	Efficacy rate as measured by serum bactericidal activity			
		A	C	Y	W-135
Menactra	2-3 years	57%	62%	84%	53%
	4-10 years	69%	81%	98%	69%
	11-18 years	100%	99%	98%	99%
	18-55 years	100%	99%	91%	97%
Menomune ACYW135	2-12 years	72%	58%	90%	82%
Menomune ACYW135	2-3 years	55%	30%	57%	26%
	4-10 years	48%	38%	84%	68%
	11-18 years	100%	99%	100%	99%
	18-55 years	99%	98%	97%	99%
Mencevax ACWY	2-5 years	90.9%	78.6%	100%	92.9%
	≥ 6 years	100%	99%	100%	100%

On the other hand, the applicant explained the status of development of vaccines other than the tetravalent meningococcal vaccines approved overseas as follows:

Concerning the status of development of a serogroup B meningococcal vaccine, two products, [redacted] ([redacted]) and [redacted] ([redacted]) are marketed in certain regions only (Cuba, Brazil, Chile, Argentina, New Zealand, etc.) to control and prevent strain-specific infections and a phase III clinical trial of [redacted] is currently ongoing overseas, but the details are unknown.

PMDA considers as follows:

According to a survey of 45 Russian patients with LCCD who experienced recurrent meningococcal infections followed for 3 to 8 years, 31 of the 45 patients were immunized with a tetravalent (serogroups A, C, Y, and W135) meningococcal vaccine and the survival rate was improved in vaccinees compared with non-vaccinees (*Vaccine*. 2003;21:4437-4447.). Thus, the efficacy of vaccination can be expected also in patients treated with eculizumab in whom the formation of C5b-9 is blocked, like LCCD patients. On the other hand, as described in "(b) Epidemiology of meningococcal infections ii) Differences in meningococcal infections between Japan and overseas," it is considered that serogroup B disease is most common in Japan (serogroup B accounted for 56.5% of isolates for 182 cases collected between 1974 and 2003; serogroup B accounted for 26.8% of isolates for 82 cases collected between April 1999 and December 2004; and serogroup B accounted for 32% of isolates for 807 cases collected between 1992 and 1996 in the US) and adverse events of Guillain-Barré syndrome etc., among recipients of a tetravalent meningococcal vaccine commonly used overseas have been reported (*MMWR Morb Mortal Wkly Rep*. 2006;55:1120-1124.). Therefore, the clinical benefits of vaccinating PNH patients in Japan need investigation.

(e) Measures against meningococcal infections in Japan

PMDA considers as follows:

Although meningococcal infections are manageable with appropriate and prompt diagnosis and treatment, as the disease can become serious/fatal, its diagnosis and treatment should begin as soon as

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possible. Therefore, prior to the use of eculizumab, adequate information must be provided to hematologists who are likely prescribers of eculizumab and patients must be educated.

PMDA asked the applicant to explain the planned measures to reduce the risks associated with meningococcal infections.

The applicant responded as follows:
In foreign countries, meningococcal vaccination as a measure to minimize the risk of developing a meningococcal infection, early detection of a suspected case of the disease, and early treatment are emphasized and healthcare professionals and patients have been educated with information materials. Patients are required to carry a Patient Safety Card and show this card to any doctor at each visit.

After the market launch in Japan, as PNH is a rare disease, eculizumab is expected to be used at medical institutions that are equivalent in size to clinical trial institutes and such medical institutions are considered to be prepared for the diagnosis and treatment of PNH and meningococcal infections. However, if eculizumab is used at a medical institution that is not sufficiently prepared to treat meningococcal infections, the medical institution should seek cooperation in advance from its neighboring medical institution that is fully prepared for the treatment of meningococcal infections. The applicant will develop materials covering the information on meningococcal infections as well as PNH for physicians and for patients and establish a system for measures against meningococcal infections in PNH patients.

In order to call attention to meningococcal infections, the applicant will state in the warnings section of the package insert that "patients should be fully informed of meningococcal infections," state in the contraindications section of the package insert that "eculizumab is contraindicated in patients with symptoms of meningococcal infection," and list "patients with a history of meningococcal infection" in the careful administration section of Precautions of the package insert.

PMDA's view on the measures against the risk of meningococcal infections is as follows:
Since the initial symptoms of meningococcal infection are largely non-specific and the disease can progress rapidly, become serious and lead to sequelae or death, adequate measures against meningococcal infections should be taken. Based on its mechanism of action, eculizumab is expected to increase the risk of infections with *Neisseria meningitidis*, patients were required to be vaccinated with meningococcal vaccine also in Study C07-001, and patients are required to be immunized with a meningococcal vaccine prior to receiving eculizumab overseas. Thus, in order to reduce the risk of meningococcal infections, meningococcal vaccination can be used as a promising measure against meningococcal infections also in Japan. However, there is currently no approved meningococcal vaccine whose efficacy and safety have been demonstrated in Japanese subjects and since i) the major

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tetravalent vaccines (serogroups A, C, Y, and W135), which are currently used overseas, are expected to have limited efficacy against serogroup B disease, which has been relatively commonly reported in Japan, ii) a reduction in the risk of developing a meningococcal infection in patients treated with eculizumab has not been confirmed for any vaccine including the serogroup B vaccines which are used in certain regions, and iii) in clinical studies in PNH patients and foreign post-marketing surveillance, in spite of vaccination, meningococcal infections including those caused by the serogroups covered by the vaccine occurred, etc., even vaccinated patients can develop a meningococcal infection.

Based on the above, although vaccination can also become a measure to minimize the risks associated with meningococcal infections, considering that even vaccination can not completely protect against the disease, other feasible measures in Japan should be taken first, regardless of meningococcal vaccination status. In order to reduce the risks associated with meningococcal infections, adequate measures against meningococcal infections should be built, adequate information on the risks and benefits of eculizumab should be provided to healthcare professionals and patients, and eculizumab should be initiated after both the healthcare professional and patient understand the risks and benefits of eculizumab. It is necessary to continue to review the need for meningococcal vaccination while collecting information actively in Japan and overseas.

4.(iii).B.(5).5) Other infections

The incidences of infections up to Week 12 in the TRIUMPH study, SHEPHERD study, and Study C07-001 and during the entire study period in studies in PNH patients (C07-001, E07-001, TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, X03-001A, E05-001) are shown in Table 35.

Table 35. Incidences of infections in Japanese and foreign clinical studies

	Up to Week 12								Entire study period					
	TRIUMPH				SHEPHERD		C07-001		TRIUMPH		Foreign clinical studies		Japanese clinical studies	
	Placebo (N = 44)		Eculizumab (N = 43)		Eculizumab (N = 97)		Eculizumab (N = 29)		Placebo (N = 44)		Eculizumab (N = 195)		Eculizumab (N = 29)	
	Incidence	N	Incidence	N	Incidence	N	Incidence	N	Incidence	N	Incidence	N	Incidence	N
Infection	68.2%	30	55.8%	24	45.4%	44	62.1%	18	77.3%	34	92.8%	181	75.9%	22
Serious infection	6.8%	3	0.0%	0	1.0%	1	3.4%	1	11.4%	5	13.8%	27	10.3%	3

TRIUMPH study (study duration, 26 weeks)
Japanese clinical studies (study duration, up to 38 weeks): C07-001, E07-001 (Interim report)
Foreign clinical studies (study duration, up to 206 weeks): TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, X03-001A, and E05-001 (Interim report)

PMDA confirmed that there were no major differences in the incidence of infections between the placebo and eculizumab groups and between Japan and overseas (up to Week 12).

PMDA asked the applicant to explain about any infection that deserves special attention other than that with *Neisseria meningitidis*.

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The applicant responded as follows:
Since eculizumab is a specific anti-C5 antibody and blocks the formation of the terminal complement complex C5b-9 without inhibiting the formation of an early complement component, C3b, which is essential for microbial opsonization and immune complex clearance, eculizumab is not considered to increase the risk of other common infections except for those caused by encapsulated bacteria (especially, *Neisseria meningitidis*) that are cleared by terminal complement components. This has been suggested also by clinical studies in PNH patients in which the overall incidence and severity of infections were similar between the eculizumab and placebo groups.

On the other hand, when opinions on the effect of eculizumab on the risk of infections other than those caused by *Neisseria meningitidis* were sought from an infection specialist and the investigators, they responded that the risk of infections with encapsulated bacteria, e.g. *Streptococcus pneumoniae* or *Haemophilus influenzae*, associated with eculizumab can not be denied. The frequency of occurrence of infections with encapsulated bacteria in patients with complement deficiencies differs depending on which complement is deficient and it has been reported that in patients with C5-9 deficiencies, the frequency of occurrence of *Neisseria sp.* infection was 57% (151 patients), the frequency of occurrence of *Streptococcus pneumoniae* infection was 0.8% (2 patients), and the frequency of occurrence of *Haemophilus influenzae* infection was 0% (0 patients) (*Clin Microbiol*. 1991;Rev 4:359-395.).

Even taking account of the above points, based on its mechanism of action, eculizumab may increase the risk of infections with encapsulated bacteria, e.g. *Streptococcus pneumoniae* or *Haemophilus influenzae*. Therefore, the applicant will develop materials calling attention to infections for patients and for healthcare professionals and list “patients with systemic infections” in the careful administration section of Precautions of the package insert.

PMDA considers as follows:
Although the submitted clinical study data showed no clear trend towards an increased risk of infections associated with eculizumab, the limited number of patients were studied and since eculizumab blocks the formation of the terminal complement complex acting on capsular polysaccharides, there may be a theoretical increased risk of infections with not only *Neisseria meningitidis*, but also *Streptococcus pneumoniae* and *Haemophilus influenzae*. In patients with C5b-9 deficiency, the incidence of *Streptococcus pneumoniae* or *Haemophilus influenzae* infection tended to be lower than the incidence of *Neisseria* infection, but it is difficult to completely deny an increased risk of infections with these bacteria.

Based on the above, there is no problem with listing “patients with infections” in the careful administration section of Precautions of the package insert. It is also necessary to develop materials for patients and for healthcare professionals to call attention to infections as a whole. In addition, it is

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necessary to collect information on the incidence, severity, and outcome of infections via post-marketing surveillance etc. and provide the information appropriately to healthcare professionals and patients if a new finding becomes available.

4.(iii).B.(5).6) Infusion reactions

Since eculizumab is an antibody preparation, administration of eculizumab may result in infusion reactions. PMDA asked the applicant to explain how to prevent/manage infusion reactions.

The applicant responded as follows:

In clinical studies, a specific guidance on how to manage infusion reactions was not provided and infusion reactions were prevented and managed at the discretion of the physician. Mild to moderate infusion reactions were reported by 1 patient in the TRIUMPH study and 3 patients in the SHEPHERD study, which resolved following interruption of eculizumab or symptomatic treatment and then the patients were able to continue treatment with eculizumab with preventive measures taken before the administration of eculizumab.

It is recommended to slow the infusion rate (up to 5 mg/min for 600 mg doses, up to 7.5 mg/min for 900 mg doses) and administer an histamine H_1 antagonist, an histamine H_2 antagonist, or a steroid, etc. At the medical institution, patients should be monitored for at least 1 hour following the completion of the infusion or patients with an infusion reaction or a history of an infusion reaction should be monitored for at least 2 hours following the completion of the infusion to ensure safety.

PMDA considers as follows:

The information on the recommended practices for the prevention and management of infusion reactions should be provided appropriately. After the market launch, the information on the development of infusion reactions, its relationship with the infusion rate, and how infusion reactions were managed and prevented should be collected and the information should be provided to the medical practice as appropriate.

4.(iii).B.(5).7) Immunogenicity and human anti-human antibodies (HAHA)

The applicant explained the immunogenicity and HAHA induction following treatment with eculizumab as follows:

The rates of the induction of HAHA in foreign clinical studies of eculizumab in PNH and non-PNH subjects are shown in Table 36. HAHA was not detected in a Japanese clinical study C07-001 and analysis has not been performed in Study E07-001 (Interim report). The presence of neutralizing antibodies has not been determined because its assay is under development.

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Table 36. HAHA response following treatment with eculizumab

	PNH clinical studies				Non-PNH clinical studies[a)]				All studies			
	Placebo		Eculizumab		Placebo		Eculizumab		Placebo		Eculizumab	
No. of patients with HAHA measurement	N = 44		N = 151		N = 206		N = 677		N = 250		N = 828	
	Incidence	N	Incidence	N	Incidence	N	Incidence	N	Incidence	N	Incidence	N
Positive HAHA response	2.3%	1	1.3%	2	5.3%	11	3.8%	26	4.8%	12	3.4%	28
Positive IgM response	–	–	0.7%	1	2.4%	5	1.0%	7	2.0%	5	1.0%	8
Positive IgG response	2.3%	1	1.3%	2	3.4%	7	3.0%	20	3.2%	8	2.0%	21
Positive IgG and IgM response	–	–	–	–	0.5%	1	0.1%	1	0.4%	1	0.1%	1

a) Clinical studies in patients with rheumatoid arthritis, idiopathic membranous glomerulonephritis, dermatomyositis, or psoriasis

Regarding safety in patients with a positive HAHA response, 1 patient each in the TRIUMPH and SHEPHERD studies developed HAHA within 26 weeks of treatment and the 1 patient in the TRIUMPH study had coagulopathy, headache, and haematoma and the 1 patient in the SHEPHERD study had pyrexia and nasopharyngitis. One patient in the placebo group in the TRIUMPH study also had a positive HAHA response, which was considered due to measurement errors or contaminants. The assay method for the measurement of HAHA has been improved and the sensitivity of the assay method used in the Japanese clinical study is higher than that used in the foreign clinical studies. As the assay reagent used in Study C07-001 will become unavailable at the end of 2010, a new assay method will be employed in January 2010.

PMDA considers as follows:

At present, the relationship between positive HAHA response and the occurrence of adverse events is unknown. After the market launch, if adverse events of infusion reactions etc. possibly related to HAHA induction occur or there is loss of efficacy of eculizumab etc., HAHA should be measured as appropriate and the information on HAHA should be collected and provided to the medical practice as appropriate.

4.(iii).B.(5).8) Safety after eculizumab discontinuation

By protecting PNH type III RBCs against intravascular hemolysis, the percentage of PNH type III RBCs increases in patients being treated with eculizumab (Based on the pooled data from the TRIUMPH and SHEPHERD studies, the percentage of PNH type III RBCs [median] was 31.7% at baseline, 46.5% at Week 8, and 51.5% at Week 26) and a serious hemolytic crisis may be induced if eculizumab is discontinued. PMDA asked the applicant to explain the clinical courses of patients who were discontinued from treatment with eculizumab and necessary measures after discontinuation.

The applicant responded as follows:

In Japanese and foreign clinical studies in PNH patients, a total of 13 patients were discontinued from eculizumab treatment due to lack of efficacy or adverse events etc. In the foreign marketing experience, 67 cases of treatment discontinuation were reported and the information after treatment discontinuation was obtained for 51 of the 67 cases. When “serious hemolysis” after treatment discontinuation is defined as a rise in LDH above the baseline level at the onset of the event and either (a) a ≥ 25%

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reduction in PNH type III RBCs within 1 week of the onset of the event, (b) Hb < 5.0 g/dL or a > 4.0 g/dL reduction in Hb within 1 week of the onset of the event, (c) the occurrence of angina pectoris or TE, (d) mental status changes, or (e) a 50% increase in serum creatinine, serious hemolysis meeting this definition has not been reported among the patients who were discontinued from eculizumab treatment in the clinical studies and marketing experience.

However, as the risk of serious hemolysis can not be denied theoretically, after the market launch, patients will be monitored for at least 8 weeks after eculizumab discontinuation to detect serious hemolysis. If serious hemolysis occurs after eculizumab discontinuation, PRBC transfusion, readministration of eculizumab, and exchange transfusion etc. should be considered.

PMDA considers as follows:
Although there has so far been no patients who developed serious hemolysis meeting the applicant's definition after eculizumab discontinuation, as the risk of a serious hemolytic crisis exists theoretically, the information on the risks associated with eculizumab discontinuation and the need for monitoring patients after eculizumab discontinuation should be provided to the medical practice appropriately. Since appropriate management after eculizumab discontinuation has not been established, it is unavoidable that the patients are treated at the physician's discretion according to individual patients' symptoms. After the market launch, the information on the clinical course and management of patients after eculizumab discontinuation should be collected and the information on the risks associated with discontinuation and management should be provided to the medical practice as appropriate.

4.(iii).B.(6) Indication
The proposed indication was "paroxysmal nocturnal hemoglobinuria (PNH)."

PMDA's view on the indication for eculizumab is as follows:
The TRIUMPH study demonstrated the superiority of eculizumab over placebo for "Hb stabilization" and "the number of PRBC units transfused" and the SHEPHERD study and Study C07-001 showed reductions in LDH from baseline. The results of these studies and subgroup analyses indicate that eculizumab reduces hemolysis in PNH patients while Hb stabilization and a reduction in the number of PRBC units transfused, which are considered clinical indicators of treatment effect, have been demonstrated in the TRIUMPH study only. Therefore, although the submitted data have shown reduction in hemolysis in PNH patients, treatment effect associated with reduction in hemolysis has been demonstrated only in the TRIUMPH study population, i.e. patients who are relatively more dependent on transfusions and whose bone marrow is relatively spared.

The effects of eculizumab on the incidence of TE, which is regarded as a clinically serious problem in PNH patients, and survival prognosis have been undefined also in the TRIUMPH study and whether

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reduction in hemolysis and improvement of anemia by eculizumab are related to the ultimate improvement in survival prognosis is unknown.

On the other hand, regarding safety, although there has been no serious problem in clinical studies, as the potential risks of eculizumab, an increased risk of infections with encapsulated bacteria, e.g. *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* and the risk of a serious hemolytic crisis after treatment discontinuation have been identified.

Therefore, eculizumab should be used in appropriate patients, fully examining treatment effects and potential risks, taking into account that eculizumab is palliative and improvement in survival prognosis has not been demonstrated at present.

Based on the above, the appropriate indication statement should be "reduction of hemolysis in patients with paroxysmal nocturnal hemoglobinuria" etc. and it should be stated that eculizumab is indicated for transfusion-dependent patients with a PNH type III RBC clone ≥ 10%, i.e. the patient population included in the clinical studies. The package insert should state that Hb stabilization and reductions in transfusion requirements have been demonstrated in the TRIUMPH study population and advise that eculizumab should be used only if the clinical benefits are considered to outweigh the potential risks.

Moreover, the following information should also be provided appropriately: the eligibility criteria for the TRIUMPH study; and the efficacy of eculizumab during an acute hemolytic crisis has not been demonstrated.

Since foreign extension studies suggest that eculizumab may reduce the incidence of TE, it is desirable to continue to collect information and investigate the incidence of TE, taking account of differences in PNH conditions between Japan and overseas.

The way of providing information on the intended population for eculizumab, e.g. the indication and precautions for indication of the package insert, will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(7) Dosage and administration

The applicant explained the rationale for the dosage regimen used in clinical studies in PNH patients and how dosage and administration was established as follows:

In a study of eculizumab in patients with rheumatoid arthritis conducted in the early phase of development, although weekly administration of 8 mg/kg completely inhibited C5 activity in the sera from all patients, when the patients were switched to once every 2 weeks administration, C5 concentrations recovered in several patients. Then, considering that 35 μg/mL of eculizumab can

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theoretically bind to all C5 in human serum and based on the results from clinical studies in patients with rheumatoid arthritis or idiopathic membranous glomerulonephritis etc., a serum eculizumab concentration of 35 μg/mL was chosen as a threshold for efficacy [see "4.(ii).B.(1). Relationship between serum eculizumab concentration and hemolytic activity"].

Since a simulation showed that the trough concentrations corresponding to the 8 mg/kg dose can be maintained at a fixed dose of 600 mg, Study C02-001 in PNH patients used fixed doses of eculizumab and a dosage regimen of 600 mg weekly for 4 doses followed by 900 mg every 2 weeks for the purpose of maintaining serum eculizumab concentrations sufficient to completely inhibit C5 activity during the study period. In order to determine the dosage regimen that maintains serum trough concentrations of ≥ 35 μg/mL based on the results from Study C02-001, a simulation was performed using model parameters obtained from 11 PNH patients to assess dosage regimens. Finally, the relationships between serum eculizumab concentration and hemolytic activity in the TRIUMPH study, SHEPHERD study, and Study C07-001 were assessed and dosage and administration for eculizumab was established.

PMDA's view on dosage and administration for eculizumab is as follows:
Although how dosage and administration was established is understood, even serum concentrations of eculizumab above 35 μg/mL do not sufficiently inhibit hemolysis in some patients [see "4.(ii).B..(1) Relationship between serum eculizumab concentration and hemolytic activity"] and as other dosage regimens have not been investigated in PNH patients, there is no sufficient information to determine whether the proposed dosage regimen has been optimized and the appropriateness of dosage and administration is questionable. However, since the pivotal clinical study of eculizumab in PNH patients, the TRIUMPH study, has confirmed the efficacy of eculizumab [see "4.(iii).B.(4) Efficacy"], Study C07-001 has suggested reduction in hemolysis at the same dosage regimen also in Japanese patients, and the safety profile of eculizumab is also tolerable, it is unavoidable to select the dosage regimen used in the TRIUMPH study and Study C07-001 etc.

Dosage and administration for eculizumab will be finalized, taking account of comments from the Expert Discussion.

4.(iii).B.(8) Use in children, pregnant women, and nursing mothers

4.(iii).B.(8).1) Pediatric use

The applicant explained the pediatric use of eculizumab as follows:
Although pediatric patients aged ≥ 12 years were eligible for a Japanese clinical study (C07-001), no patients aged ≥ 12 and < 18 years were enrolled into the study.

In the foreign marketing experience, 34 children were treated with eculizumab (as of August 24, 2009), including 17 PNH patients, 14 patients with atypical hemolytic uremic syndrome, 1 patient with bone

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marrow transplant rejection, 1 renal transplant recipient, and 1 patient with membranoproliferative glomerulonephritis II. Adverse events reported by at least 2 patients were headache only (2 patients) and there were no major differences in the safety profile between pediatric and adult patients.

Meanwhile, for the development of eculizumab for pediatric use, a clinical study to evaluate the pharmacokinetics, pharmacodynamics, and safety of eculizumab in about 6 to 8 pediatric and adolescent patients with PNH (2-17 years) is scheduled to be initiated in the US and Europe in 2009 (Study M07-005), but there is no plan to enroll Japanese patients. Clinical studies in adolescent patients with atypical hemolytic uremic syndrome are ongoing in the US and Europe. A study in pediatric patients with atypical hemolytic uremic syndrome is also under planning and the participation of Japanese patients is under consideration.

PMDA concluded as follows:
There is very limited clinical experience with eculizumab in pediatric patients at present. Although the majority of patients are adults based on the age of onset of PNH, as there are also pediatric patients, it is preferable to develop eculizumab for Japanese pediatric patients as well.

PMDA is currently asking the applicant about the seriousness of adverse events and their causal relationship to eculizumab in the foreign post-marketing surveillance and any plan of a clinical study in Japanese pediatric patients with PNH.

4.(iii).B.(8).2) Use during pregnancy or lactation
PMDA asked the applicant to explain the use of eculizumab during pregnancy in clinical studies and foreign post-marketing surveillance.

The applicant responded as follows:
Reported pregnancies from patients on eculizumab during clinical studies and post-marketing surveillance (up to August 15, 2009) are as shown in Table 37.

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Table 37. Reported pregnancies during clinical studies and post-marketing surveillance

		Actions taken and Outcome
Clinical studies	E02-004 (Rheumatoid arthritis)	The patient discontinued eculizumab treatment and elected to terminate her pregnancy.
	SHEPHERD	The patient discontinued eculizumab treatment and elected to terminate her pregnancy.
	TRIUMPH	The patient discontinued eculizumab treatment and delivered a healthy baby by cesarean section at 39 weeks of gestation.
	E05-001	The patient discontinued eculizumab treatment and delivered a healthy baby by cesarean section at 38 weeks of gestation.
	E05-001	The patient discontinued eculizumab treatment and had a normal delivery with a healthy baby at 39 weeks of gestation.
	C02-001 and E05-001	The patient continued eculizumab treatment and delivered a healthy baby at 38 weeks of gestation (probably, a normal delivery)
Post-marketing surveillance		The patient discontinued eculizumab treatment, but recommenced due to hemolysis during pregnancy and delivered healthy babies by cesarean section (twins). (The patient developed a post-partum hemorrhage due to persistent retained products of conception.)
		The patient continued eculizumab treatment and had a spontaneous abortion at 8-9 weeks of gestation.
		The patient discontinued eculizumab treatment, but recommenced at 30 weeks of gestation and delivered healthy babies by cesarean section at 36 weeks of gestation (twins).
		The patient discontinued eculizumab treatment and elected to terminate her pregnancy due to severe AA, severe thrombocytopenia, and severe neutropenia associated with Escherichia sepsis. (Autopsy revealed no fetal abnormalities and the patient underwent allogeneic bone marrow transplantation 27 days after termination of pregnancy.)
		The patient continued eculizumab treatment and had a normal delivery with a healthy baby.
		The patient continued eculizumab treatment and had a preterm cesarean delivery at 27 weeks of gestation.
		Whether eculizumab treatment was continued or not is unknown. Due to intestinal ischaemia followed by knee pain, pneumonia, septic shock, and respiratory failure, the patient underwent a cesarean section at 27 weeks of gestation, but her baby died.
		The patient continued eculizumab treatment and was in her first month of gestation as of March 2009.
		The patient discontinued eculizumab treatment and was in the first trimester as of March 2009.
		The patient discontinued eculizumab treatment, but will recommence in the third trimester. The patient was in her 7th week of gestation as of March 2009.
		The patient continued eculizumab treatment and had a spontaneous abortion.
		Whether eculizumab treatment was continued or not is unknown. The patient had a therapeutic abortion.
		The patient continued eculizumab treatment and was in her 8th to 10th week of gestation as of April 2009.
		The patient continued eculizumab treatment and had a preterm cesarean delivery at 27 weeks of gestation.

It has been reported that PNH patients who received eculizumab and low-molecular-weight heparin during the third trimester and the perinatal period and after delivery had no adverse events from Week 30 of gestation to 3 months post-partum (*Leuk Res*. 2009;33:e4-e5.).

Although it is unknown whether eculizumab is excreted into human milk, as IgG is considered to be excreted in human milk, the possibility that eculizumab is excreted into human milk can not be ruled out and in accordance with the package insert in Europe, it will be stated in the package insert that "breast-feeding should be discontinued during treatment and up to 5 months after treatment."

PMDA considers as follows:

There is very limited clinical experience with eculizumab in pregnant women. Based on the currently available information, fetal effects are unknown.

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It is necessary to call attention to potential excretion in milk. Meanwhile, although the maximum $t_{1/2}$ (estimated by an analysis with a 1-compartmental model) in the TRIUMPH study, SHEPHERD study, Study E05-001, and Study C07-001 (566.9 hours [23.6 days]) indicates that eculizumab is almost completely eliminated from blood at 5 months after treatment, there is no definitive basis for 5 months of discontinuation of breast-feeding. Therefore, it is unnecessary to specify the period of discontinuation in the package insert.

Eculizumab is intended for chronic use and may be used in pregnant women or nursing mothers after the market launch. Thus, it should be advised that the information is limited as described above and eculizumab may be excreted into milk and it is necessary to collect information on the effects of eculizumab on pregnant women and fetuses and nursing mothers and babies and appropriately provide the information to the medical practice as needed.

4.(iii).B.(9) Post-marketing surveillance etc.

The applicant presented an outline of post-marketing surveillance (draft) as shown in Table 38.

Table 38. Outline of post-marketing surveillance plan (draft)

Objectives	**Detection of unknown and serious adverse drug reactions** **Long-term safety and efficacy** **Effects of eculizumab on the course of PNH (the course and prognosis etc. of the disease)**
Survey period	**8 years (≥ 3 years as a survey of long-term use)**
Planned number of cases	**All treated patients**
Target patient population	**PNH patients**
Major items to be investigated	**・ Patient background (medical history [TE, others], underlying disease [AA, MDS, others], PNH duration, complications [major vascular events, hepatic impairment/renal impairment, others])** **・ The status of administration of eculizumab (including the reason for discontinuation)** **・ Concomitant medications (steroids, immunosuppressants, anticoagulants, others)** **・ Adverse events** **・ Efficacy (change of LDH, improvement in QOL [fatigue, abdominal pain, dyspnoea, dysphagia], change in Hb, the number of PRBC units transfused)** **・ Clinical course up to 8 weeks after treatment discontinuation (the development of a hemolytic crisis, etc.)** **・ Significant events including malignant tumors (meningococcal infection, infections leading to serious outcomes such as hospitalization and death, death, serious hemolysis after discontinuation, pregnancy, TE, malignant tumors)** **[Priority item]** **・ Development of infections**

As an epidemiological study, the PNH registry is run by Alexion, the US, which will be incorporated into post-marketing surveillance after necessary modifications.

PMDA considers as follows:

Besides the above, the information regarding the following (a) to (c) should also be collected and monitoring after treatment discontinuation and the development of TE should be treated as priority items. As eculizumab is an orphan drug and there is very limited clinical experience with eculizumab in Japan, as a condition for approval, the applicant is required to conduct a post-marketing survey covering all treated patients. Post-marketing commitments will be finalized, taking account of comments from the Expert Discussion.

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An overview of the global PNH registry is currently being requested.

(a) PNH type III RBC clone over time
(b) Information on HAHA
(c) Safety of eculizumab in children and pregnant women/nursing mothers

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based compliance inspection and data integrity assessment was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, there were no particular problems. Thus, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

GCP on-site inspection was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application (Study C07-001, 5.3.5.2-6 and Study E07-001, 5.3.5.2-7). As a result, failure to operate the IRB in accordance with the SOP (in response to the reports of adverse drug reactions etc. notified by the sponsor, the appropriateness of continuing the clinical trial and amendments to the written information were reviewed through expedited review procedures) and protocol deviations (a chest X-ray had not been performed in some subjects) were found at some clinical trial sites. Furthermore, there were imperfections in the sponsor's monitoring report on the operation of the IRB regarding the above-mentioned reports of adverse drug reactions etc., but PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application dossier.

IV. Overall Evaluation

Based on the submitted data, the efficacy of eculizumab in patients with PNH has been demonstrated and its safety is acceptable in view of its observed benefits. Since eculizumab is an anti-C5 antibody and offers a new treatment for patients with PNH, it has clinical significance. PMDA considers that the points listed below need to be further reviewed.

Eculizumab may be approved if it can be concluded based on comments from the Expert Discussion that there are no particular problems.

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- Data subject to evaluation
- Efficacy
- Safety
- Measures against meningococcal infections
- Indication
- Dosage and administration
- Post-marketing commitments

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Review Report (2)

February 18, 2010

I. Product Submitted for Registration

[Brand name]	Soliris for Intravenous Infusion 300 mg (Note: The proposed Japanese brand name has been modified.)
[Non-proprietary name]	Eculizumab (Genetical Recombination)
[Name of applicant]	Alexion Pharma K.K.
[Date of application]	March 31, 2009

II. Content of the Review

The Expert Discussion and subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined below.

The expert advisors for the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

(1) Quality

As a result of the following reviews including the questions asked to the applicant, PMDA concluded that the quality of the drug product to be marketed is adequately controlled.

(1).1) Evaluation of disulfide bond heterogeneity

Although it is understood that it is difficult to control disulfide bond variants quantitatively, PMDA asked the applicant to explain the need to control the heterogeneity of these variants, taking account of the possible effects of these variants on the safety and efficacy of eculizumab.

The applicant responded as follows:

Since disulfide bond variants are considered to be present also in the previous lots, based on the previous clinical studies and foreign clinical experience, disulfide bond variants are unlikely to affect the safety and efficacy of eculizumab. However, as the possibility that differences in disulfide bond heterogeneity affect the safety and efficacy of eculizumab can not be ruled out, a quantitative assay for determining the relative abundance of disulfide bond variants will be developed and the acceptance criteria will be established.

PMDA accepted the applicant's response that although no specification for disulfide bond variants is set

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at present, an assay will be developed to control disulfide bond variants, taking account of previous clinical experience and the clinical need for eculizumab.

(1).2) Control of immunogenic glycans

Since eculizumab contains fucosylated biantennary *N*-linked oligosaccharides bearing immunogenic Neu5Gc or αGal (including Galα1-3Gal), PMDA asked the applicant to explain the influences of glycan structure and its heterogeneity on the safety of eculizumab and the need to include the Neu5Gc content in the specification and provide a justification for the specification limit for the αGal content.

The applicant responded as follows:

Glycans in the Fc region are considered to be inside eculizumab and not to be recognized by molecules in the body. No adverse events of anaphylactic shock etc. following the administration of eculizumab have been reported so far. The number of patients who developed HAHA was limited (see Review Report (1) "4.(iii).B.(5).7) Immunogenicity and human anti-human antibodies (HAHA)"). Therefore, these glycans are unlikely to affect the immunogenicity of eculizumab.

The glycan structure of eculizumab and its heterogeneity can be controlled by the drug substance specifications for glycan profile and the upper limit for the αGal content and there is no plan to include the Neu5Gc content in the specification at present. However, if adverse events considered associated with glycans are reported in the safety information in future, the acceptance criteria for glycans will be reviewed. The current specification limit for the αGal content was established based on the results of lot analyses (10 drug substance lots produced by July 2005) that were available at the time of setting the specification in the US/Europe (July 2008). Whether the specification limit should be reviewed will be determined, based on the analytical results on recently manufactured lots.

PMDA concluded that although the immunogenicity of Neu5Gc and Galα1-3Gal has not completely been defined at present, based on previous clinical experience, glycan structure is unlikely to affect the safety of eculizumab as long as glycosylation is controlled by the current specifications, and accepted the response. However, depending on future safety information, it is necessary to consider reviewing the acceptance criteria for glycans, etc.

(1).3) C5 binding assay

PMDA asked the applicant to provide a justification for the specification limits, taking account of the potencies of the clinical formulation and the proposed commercial formulation analyzed by the new assay employed in [redacted] 20[redacted] and clinical experience.

The applicant responded as follows:

The potencies of [redacted] lots of the proposed commercial formulation analyzed by the new assay were

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871,237 to 1,149,500 BU/mg, which were within the current specification limits (700,000-1,300,000 BU/mg) and no new adverse events associated with a switch to the proposed commercial formulation analyzed by the new assay have been reported so far. Therefore, the performance of the new assay is considered to be comparable to that of the old assay and the same specification limits can be used. As analytical results are limited at present, whether the specification limits should be reviewed will be determined, based on future manufacturing experience.

PMDA accepted the response.

(1).4) Drug product specification

The applicant explained as follows:

Though IEF and hemolytic assay are included in the drug product specification in the US and Europe, IEF and hemolytic assay are not included in the drug product specification in Japan because it is difficult to perform these tests in Japan for the following reasons:

- Skills and experience are required for performing the tests.
- It is difficult to maintain the precision and reproducibility of the tests with limited drug product lots imported to Japan and perform the tests consistently.
- It is uncertain if the reagents used in hemolytic assay (chicken erythrocytes, etc.) can be procured in Japan.

The quality of the drug product to be marketed in Japan can be assured by the results of IEF and hemolytic assay performed at the time of its release. The drug product after release testing is stored and shipped under adequate control and shipping validation and quality assessments of the drug product before and after shipment have demonstrated that the quality of the drug product is unaffected.

PMDA requested the applicant to take the following actions:

If IEF and hemolytic assay can not be included in the drug product specification in Japan, these tests that are performed overseas should be performed as in-process controls in the drug product manufacturing process, for the time being. Since IEF can separate differently charged species, it is preferable to include IEF in the specification. Thus, a system under which IEF can be performed should be established promptly and IEF should be included in the drug product specification. According to the results of validation of hemolytic assay presented in the application dossier, the assay results tend to be inherently variable. From this and other reasons, it is understood that it is technically difficult to perform hemolytic assay in Japan. However, since hemolytic assay can assess the ability and capacity of eculizumab to achieve a biological effect (a pharmacodynamic effect), including hemolytic assay in the drug product specification in Japan in future should be considered.

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The applicant responded as follows:
For the time being, the both tests will be performed as in-process controls. A system under which IEF can be performed in Japan will be established promptly after approval and IEF will be included in the drug product specification. Furthermore, the possibility of performing hemolytic assay in Japan will continue to be reviewed.

PMDA accepted the response.

(1).5) Conformity of raw materials of animal origin to the Standard for Biological Ingredients
US-sourced BSA that does not conform to the Standard for Biological Ingredients is used as a medium component in the preparation and preservation of the MCB and WCB and for cell culture. PMDA asked the applicant to explain the risk assessment of BSA, the benefit of eculizumab, and the timing of switching. The Standard for Biological Ingredients does not apply to US-sourced BSA that is used in the preparation and preservation of the MCB and WCB in accordance with "Partial Revision of the Standard for Biological Ingredients" (PFSB Notification No. 0705001 dated July 5, 2004).

The applicant responded as follows:
With respect to the risk of transmissible spongiform encephalopathy (TSE) infection associated with the use of this raw material, "geographical risk and risk of the specific parts used for raw materials of drugs etc." and "risks associated with treatment in the product manufacturing process and product usage" ("Handling of Risk Assessment etc. in Partial Change Applications for Drugs, Medical Devices, etc., Using Bovine Derived Raw Materials etc." [PFSB/ELD Notification No. 0801001 and PFSB/SD Notification No. 0801001 dated August 1, 2003]) were assessed. As a result, the total risk assessment score was "less than -3," which met a threshold to provide a certain safety assurance and the risk of TSE infection associated with the use of eculizumab is very low. At present, there is no effective existing therapy for the hemolysis of PNH and the medical benefit of eculizumab is high. Switching to New Zealand-sourced BSA is currently under consideration and a partial change application for switching to BSA sourced from another country will be filed by the 4th quarter of 2011.

PMDA considers as follows:
Although the risk of TSE infection associated with the use of eculizumab can not be excluded, the result of risk assessment indicates that its risk is very low. There is no effective therapy for the hemolysis of PNH at present and as eculizumab stabilized Hb levels and reduced transfusion requirements by reducing hemolysis in the TRIUMPH study, eculizumab is expected to become an effective therapy for the hemolysis of PNH (However, it is necessary to select patients carefully in view of safety concerns [e.g. theoretical increase in the risk of infection with *Neisseria meningitidis* etc.]). Therefore, the medical usefulness of eculizumab should outweigh the risks associated with the use of this raw material.

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Based on the above, as this case falls under 4-1-(5) of the Standard for Biological Ingredients, there is no need to withhold the approval of eculizumab until the raw material is switched to that sourced from countries where no bovine spongiform encephalopathy (BSE) has been reported. However, it should be stated in the package insert that the risk of TSE infection can not be excluded and patients should be fully explained about this risk prior to the use of eculizumab.

The above conclusion is based on the premise that the product will be manufactured using the raw material that conforms to the Standard for Biological Ingredients as soon as it becomes possible.

(2) Results from clinical pharmacology studies

PMDA reviewed the applicant's response to the question as follows and considered that there are no major differences in the effect of eculizumab between Japan and overseas.

(2).1) Hemolytic activity over time in clinical studies

The measured hemolytic activity over time in the C02-001, TRIUMPH, SHEPHERD, and C07-001 studies are shown in Figure 4. There were no major differences in the results.

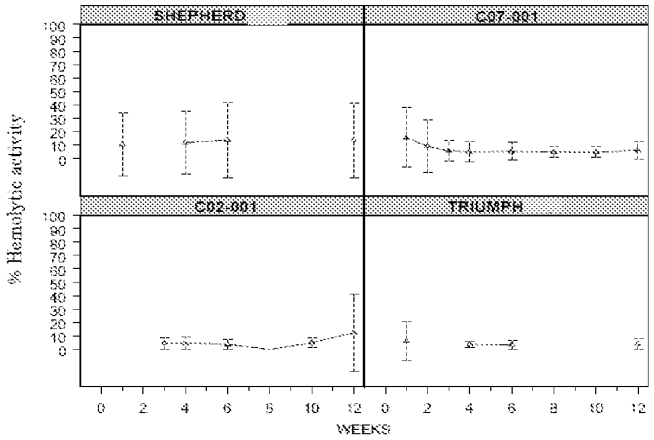

Figure 4. Hemolytic activity over time in clinical studies (C02-001, TRIUMPH, SHEPHERD, C07-001)

(3) Data subject to evaluation

For the review of the application, PMDA decided to position the foreign TRIUMPH study as the pivotal study and use the foreign SHEPHERD study and the Japanese C07-001 study as supporting efficacy and safety data, taking fully into consideration epidemiological differences in PNH between Japan and overseas and differences in patient background etc. among the studies, for evaluations, for the following reasons: since PNH is a rare disease, it is difficult to conduct a confirmatory clinical study in Japan alone; and since PNH is an acquired genetic disorder and eculizumab is an anti-C5 antibody, it is

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inferred from the pathology of the disease and the mechanism of action that eculizumab is insensitive to ethnic factors.

The expert advisors made the following comment and supported the PMDA's decision:

- While TE is a clinical problem for PNH patients in the US and Europe, fewer patients develop TE and hematopoietic failure and renal impairment are more likely to become problems in Japan, etc., indicating differences in the clinical symptoms and causes of death of PNH between Japan and overseas. Therefore, what clinical significance is ultimately expected of hemolysis inhibition by eculizumab may be different between Japan and the US/Europe. However, as long as the efficacy of eculizumab is evaluated based on hemolysis inhibition, it is inferred that eculizumab is insensitive to ethnic factors.

(4) Efficacy

The following conclusions by PMDA were supported by the expert advisors:

Choosing "Hb stabilization" and "the number of PRBC units transfused" as the primary efficacy endpoints for the TRIUMPH study was appropriate for demonstrating the clinical significance of eculizumab as PNH patients primarily present with progressive anemia due to chronic intravascular hemolysis. Although the effects of eculizumab on the development of TE and survival prognosis etc. are undefined at present, as eculizumab was shown to be effective in maintaining Hb at levels that did not necessitate transfusions and reducing transfusion requirements in the TRIUMPH study, the clinical significance of hemolysis inhibition by eculizumab was demonstrated. The SHEPHERD study and Study C07-001 demonstrated reduction in hemolysis as indicated by changes of LDH, but have not assessed treatment effect, e.g. Hb stabilization.

PMDA reviewed the applicant's responses to the questions about efficacy as follows and considered that the efficacy of eculizumab can be expected.

(4).1) Influence of body weight

The applicant responded that there was no particular trend for the influence of body weight on the efficacy and safety of eculizumab in the TRIUMPH study, SHEPHERD study, and Study C07-001 and PMDA accepted the response.

(4).2) Long-term efficacy

LDH levels over time and transfusion requirements over time based on pooled analyses of the submitted foreign clinical studies (TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, X03-001A, E05-001) are shown in Tables 39 and 40, respectively. PMDA considered that it was suggested that continued treatment with eculizumab resulted in sustained decrease in hemolysis up to 24 months after start of treatment.

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Table 39. LDH levels over time in foreign clinical studies[a)]

	Baseline	3 months	6 months	9 months	12 months	15 months	18 months	24 months
N	151	150	147	107	102	11	11	11
LDH (U/L)	2238.0 [511.8, 8600.0]	364.8 [126.7, 2594.0]	290.7 [111.7, 5117.0]	299.7 [109.3, 1192.3]	285.4 [103.0, 821.0]	528.5 [155.5, 755.0]	500.5 [157.5, 683.0]	532.0 [141.0, 740.0]

Median [Min., Max.]
a) TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, X03-001A, E05-001

Table 40. Transfusion requirements over time in foreign clinical studies[a)]

	Baseline[b)]	0-3 months	3-6 months	6-9 months	9-12 months	12-15 months	15-18 months	21-24 months
N	151	150	147	107	102	11	11	11
No. of units transfused	3.3 [0.0, 16.5]	0.0 [0.0, 16.0]	0.0 [0.0, 14.0]	0.0 [0.0, 12.0]	0.0 [0.0, 16.0]	2.0 [0.0, 6.0]	0.0 [0.0, 3.0]	0.0 [0.0, 5.0]

Median [Min., Max.]
a) TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, X03-001A, E05-001
b) The number of units transfused in the past 1 year divided by 4

(4).3) Lack of efficacy

PMDA asked the applicant about the details of 14 cases of lack of efficacy reported from foreign post-marketing surveillance (Table 32).

The applicant explained as follows:

Among the 14 patients diagnosed with lack of efficacy, 4 patients continued treatment, 8 patients discontinued treatment, and 2 patients had unknown status regarding treatment continuation. The reasons for classifying patients as lack of efficacy included complications (worsening of AA or MDS, etc.), inadequate doses, a hemolytic crisis associated with a reduced dose of steroids, no reduction in transfusion requirements, and death due to sepsis. Changes in LDH levels were reported in 6 of the 14 patients and treatment with eculizumab resulted in reductions in LDH levels in all 6 patients.

PMDA considered as follows:

Concerning the information on the patients reported as lack of efficacy via foreign post-marketing surveillance, there were patients classified as lack of efficacy in the judgment of the attending physician despite the fact that LDH levels were reduced following treatment with eculizumab. In the Japanese clinical study (C07-001), 2 patients were classified as lack of efficacy as LDH levels were not reduced. Based on these findings, it is necessary to continue to collect information on patients classified as lack of efficacy also after the market launch and investigate the reasons for classifying patients as lack of efficacy and the causes for lack of efficacy.

(5) Safety

(5).1) Overall safety of eculizumab

PMDA concluded as follows:

The incidences of adverse events of headache, nasopharyngitis, back pain, and fatigue tended to be higher in the eculizumab group than in the placebo group in the TRIUMPH study[††††] and attention

[††††] In response to the request from PMDA, the applicant discussed that the causes for higher incidences of nasopharyngitis, back pain, and fatigue in the eculizumab group than in the placebo group in the TRIUMPH study are unknown.

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should be paid to the possible occurrence of headache, especially during the early phase of treatment. There were no serious adverse events unique to the eculizumab group or no adverse events unique to long-term treatment. Thus, adverse events associated with eculizumab are tolerable.

On the other hand, based on its mechanism of action, eculizumab may increase the risk of infections with encapsulated bacteria (especially, *Neisseria meningitidis*) that are cleared by terminal complement components and attention should be paid to infections, especially meningococcal infection [see "(5).2) Meningococcal infections"]. Furthermore, since there is a concern about the development of a serious hemolytic crisis after eculizumab discontinuation due to the accumulation of PNH type III RBCs in patients being treated with eculizumab, the information on the risks associated with discontinuation of eculizumab and the need for monitoring patients after eculizumab discontinuation should be provided appropriately to the medical practice.

The above conclusions by PMDA were largely supported by the expert advisors, but the following comments were raised from some expert advisors.

- Although the incidence of headache was higher in the eculizumab group, as headache occurs also as an initial symptom of meningococcal infection, it may become necessary to differentiate the causes of headache. How headache is managed overseas should be checked, an appropriate approach to headache should be considered, and information should be provided to the medical practice.
- Theoretically, the risk of infections with not only *Neisseria meningitidis*, but also other encapsulated bacteria, e.g. *Streptococcus pneumoniae* and *Haemophilus influenzae*, may be increased. The status of vaccination against *Streptococcus pneumoniae* and *Haemophilus influenzae* in foreign countries where eculizumab has been approved should be checked and then the incidence of infections among eculizumab-treated patients in Japanese and foreign marketing experience should continue to be investigated and necessary actions should be taken.
- Theoretically, the risk of a serious, acute hemolytic crisis is increased after eculizumab discontinuation and adequate caution should be provided. For the cases of treatment discontinuation, the information after discontinuation should be collected.

PMDA asked the applicant to explain how to differentiate a headache associated with eculizumab from a headache caused by *Neisseria meningitidis*.

The applicant explained as follows:
It is difficult to specifically differentiate a headache associated with eculizumab from a headache caused by meningococcal infection if there are no other symptoms. Meanwhile, differentiation is considered possible in most cases based on (a) the presence or absence of other symptoms of meningococcal infection (pyrexia, nausea/vomiting, neck or back stiffness, rash, mental confusion, severe myalgia

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accompanied by flu-like symptoms, photophobia) or (b) antipyretic analgesics are ineffective against a headache caused by meningococcal infection. Performing head tapping test, jolt accentuation test, vascular compression test, nerve compression test, muscle compression test, and tension sign tests etc. as appropriate and determining the presence or absence of symptoms of meningeal irritation due to bacterial meningitis should also be considered in order to diagnose the cause of headache.

However, basically, at the discretion of specialists, tests for infections (blood culture and lumbar puncture etc.) will be performed and antibiotics will be administered.

PMDA asked the applicant to explain the status of vaccination against *Streptococcus pneumoniae* and *Haemophilus influenzae* in foreign countries where eculizumab has been approved.

The applicant explained as follows:
The status of vaccination in a Japanese clinical study (C07-001) and foreign clinical studies (TRIUMPH, SHEPHERD) is shown in Table 41.

According to the foreign post-marketing data as of January 31, 2010 (1700 case reports), about 1% of patients were vaccinated against *Streptococcus pneumoniae* or *Haemophilus influenzae*, or both.

Table 41. Status of vaccination against *Streptococcus pneumoniae* and *Haemophilus influenzae* in Japanese and foreign clinical studies (TRIUMPH, SHEPHERD, C07-001)

Clinical study		No. of patients	No. of vaccinated patients	
			Streptococcus pneumoniae	*Haemophilus influenzae*
TRIUMPH	Eculizumab	43	0	2
	Placebo	44	0	2
SHEPHERD		97	2	22
C07-001		29	0	0

PMDA considers as follows:
Headache is an adverse event with a high incidence as the incidence of headache in patients treated with eculizumab was 51.3% (100 of 195 patients) in foreign clinical studies (TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, X03-001A, E05-001) and 58.6% (17 of 29 patients) in the Japanese clinical study (C07-001). Differentiating a headache as an adverse drug reaction from a headache caused by meningococcal infection is important and patients must understand that they should contact their doctors immediately if headache occurs during treatment with eculizumab. Adequate information on differentiation of headaches should be provided to patients and healthcare professionals.

Taking account of the overseas situation, there seems no need to mandate vaccination against *Streptococcus pneumoniae* and *Haemophilus influenzae* at present. However, as treatment with eculizumab may theoretically increase the risk of infections with *Streptococcus pneumoniae* and *Haemophilus influenzae*, physicians should be alerted to these infections and vaccination against *Streptococcus pneumoniae* and *Haemophilus influenzae* should be considered as appropriate, at the

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discretion of the physician.

Based on the above discussion, PMDA instructed the applicant to appropriately provide information and issue an alert, based on the comments from the expert advisors, via the package insert and information leaflet. The applicant responded appropriately and PMDA accepted it.

(5).2) Meningococcal infections

PMDA concluded as follows:

Based on its pharmacological action, eculizumab may increase the risk of developing a meningococcal infection. As part of measures against meningococcal infections, patients are required to be immunized with a meningococcal vaccine prior to the use of eculizumab in the US/Europe (eculizumab is contraindicated in unvaccinated patients) and patients were required to be immunized with a meningococcal vaccine also in the TRIUMPH study, SHEPHERD study, and a Japanese clinical study (C07-001). Meningococcal vaccination can be used as a promising safety measure also in Japan. However, there is currently no approved meningococcal vaccine whose efficacy and safety have been demonstrated in Japanese subjects and the major tetravalent vaccines (serogroups A, C, Y, and W135), which are currently used overseas, are expected to have limited efficacy against serogroup B disease, which has been relatively commonly reported in Japan, etc. Thus, it is necessary to continue to review the need for meningococcal vaccination while collecting information actively from Japan and overseas. In order to reduce the risks associated with meningococcal infections, adequate measures against meningococcal infections, e.g. early detection and early initiation of treatment with antibiotics, should be taken. Adequate information on the risks and benefits of eculizumab should be provided to healthcare professionals and patients and eculizumab should be initiated after both the healthcare professional and patient understand the risks and benefits of eculizumab.

The expert advisors made the following comments on the above conclusions by PMDA:

- PNH patients with decreased white blood cells due to AA or MDS are susceptible to infections and especially among Japanese PNH patients, the proportion of patients with AA or MDS is relatively high. Therefore, if they develop a meningococcal infection, the disease is likely to become serious.
- Even if the major tetravalent meningococcal vaccines, which are currently used overseas, have limited efficacy in Japan, these vaccines can be expected to be effective in some patients. Therefore, at least, an environment where meningococcal vaccination is available upon request should be created.
- The regulatory authorities should guide the industry to proceed with the development of meningococcal vaccine in Japan.
- Since meningococcal infections will be manageable in most cases with early detection and appropriate early treatment, adequate information on infections, e.g. treatment with antibiotics,

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should be provided and cooperation with a medical institution prepared to treat infections is needed.

- Prior to the use of eculizumab, adequate information on the risk of meningococcal infections should be provided to healthcare professionals. Patients should also be fully informed of the risk and their consent should be obtained before the initiation of treatment.

Taking account of the comments from the expert advisors, PMDA asked for the applicant's view on the provision of information on meningococcal infections and meningococcal vaccination after marketing approval of eculizumab.

The applicant explained meningococcal vaccination prior to the use of eculizumab as follows:
(a) In cooperation with a foreign vaccine manufacturer ([redacted]), a clinical trial of a tetravalent meningococcal vaccine (serogroups, A, C, Y, and W135) (brand name, [redacted]) intended for the prevention of meningococcal infections in PNH patients will be initiated as soon as possible in Japan.
(b) The following procedures will be followed until meningococcal vaccine receives marketing approval in Japan.

i) The physician identifies a PNH patient considered to require treatment with eculizumab and provides the patient with the information on the risk of developing a meningococcal infection associated with eculizumab, the prevention and treatment methods of meningococcal infections, and its efficacy and safety.
ii) Ask the patient if he/she wishes to receive meningococcal vaccine.
iii) If the patient wishes to receive meningococcal vaccine, ask the patient if he/she wishes to participate in a clinical trial of meningococcal vaccine. If the patient wishes not to participate in the clinical trial of meningococcal vaccine or the medical institution treating the patient does not participate in the clinical trial of meningococcal vaccine, provide the information on the private import method or the health facilities providing meningococcal immunization services for overseas travelers, etc.‡‡‡‡ to the medical institution to explain to the patient.
iv) Deliver eculizumab after confirming that the patient has received meningococcal vaccine.
v) If the patient wishes not to receive meningococcal vaccine, receive a written pledge from the patient stating that the patient fully understands the risk of meningococcal infections and starts treatment with eculizumab, before delivery of eculizumab.

PMDA considers as follows:
In light of the current situation where there is no approved meningococcal vaccine in Japan, it is unavoidable at present to follow the procedures proposed by the applicant.

Since even meningococcal vaccination can not completely protect against meningococcal infections, as

‡‡‡‡ In cooperation with the Japanese Society of Travel and Health.

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feasible measures to reduce the risks associated with meningococcal infections in Japan, measures against meningococcal infections, e.g. cooperation with a medical institution prepared to treat infections, early detection, and early initiation of treatment with antibiotics, should be built, adequate information on the risks and benefits of eculizumab should be provided to healthcare professionals and patients, and eculizumab should be initiated after both the healthcare professional and patient understand the risks and benefits of eculizumab.

The applicant responded that they will address accordingly and PMDA accepted it.

PMDA instructed the applicant to prepare the details of information leaflets before the market launch and the applicant responded that they will do so.

(5).3) Results of foreign post-marketing surveillance

In response to the request from PMDA, the applicant explained differences in observed adverse events between clinical studies and post-marketing surveillance as follows:

Hb decreased was observed in the foreign post-marketing surveillance only. This is possibly because PNH-related laboratory findings may have been regarded as adverse events in the post-marketing surveillance but were not reported as adverse events in the clinical studies. Thus, the differences in observed serious adverse events are considered to have no clinically relevant significance.

PMDA considers as follows:

Although no particular events were reported at a markedly higher incidence in the foreign post-marketing surveillance than in the clinical studies, it is necessary to continue to collect information on the cases of lack of efficacy, e.g. Hb decreased, via post-marketing surveillance etc. and provide the information to the clinical practice as appropriate.

(5).4) Adverse events in pooled Japanese and foreign clinical studies

In response to the request from PMDA, the applicant presented adverse events occurring in ≥ 10.0% of patients in either group during the entire treatment period in all clinical studies as shown in Table 42 and serious adverse events occurring in at least 2 patients in either group during the entire treatment period as shown in Table 43.

PMDA confirmed the following:

Even when adverse events occurring up to Week 12 in the TRIUMPH study, SHEPHERD study, and Study C07-001 and foreign clinical studies (TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, E05-001) were pooled with other clinical studies, there were no significant major differences compared with Tables 29 and 30 and no problematic adverse events or serious adverse events were reported in Japan compared with overseas.

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Table 42. Adverse events occurring in ≥ 10.0% of patients in either group during the entire treatment period in clinical studies (Pooled analysis)

	Foreign clinical studies				Japanese clinical studies			Foreign clinical studies				Japanese clinical studies	
	Placebo (N = 44)		Eculizumab (N = 195)		Eculizumab (N = 29)			Placebo (N = 44)		Eculizumab (N = 195)		Eculizumab (N = 29)	
	Incidence	N	Incidence	N	Incidence	N		Incidence	N	Incidence	N	Incidence	N
Overall	90.9%	40	99.0%	193	100.0%	29	Arthralgia	9.1%	4	15.9%	31	3.4%	1
Headache	27.3%	12	51.3%	100	58.6%	17	Cough	9.1%	4	15.9%	31	3.4%	1
Nasopharyngitis	18.2%	8	42.1%	82	58.6%	17	Dizziness	11.4%	5	15.4%	30	3.4%	1
Nausea	11.4%	5	25.1%	49	20.7%	6	Pain in extremity	2.3%	1	14.4%	28	3.4%	1
Diarrhoea	11.4%	5	22.1%	43	17.2%	5	Influenza like illness	2.3%	1	11.3%	22	3.4%	1
Pyrexia	4.5%	2	16.9%	33	13.8%	4	Myalgia	2.3%	1	10.8%	21	3.4%	1
Anaemia	2.3%	1	3.6%	7	13.8%	4	Pharyngolaryngeal pain	9.1%	4	14.4%	28	0.0%	0
Conjunctival haemorrhage	0.0%	0	1.0%	2	13.8%	4	Abdominal pain	11.4%	5	12.8%	25	0.0%	0
Oedema	0.0%	0	0.0%	0	13.8%	4	Insomnia	6.8%	3	11.8%	23	0.0%	0
Upper respiratory inflammation	0.0%	0	0.0%	0	13.8%	4	Constipation	4.5%	2	11.8%	23	0.0%	0
Vomiting	11.4%	5	16.4%	32	10.3%	3	Viral infection	11.4%	5	11.3%	22	0.0%	0
Upper respiratory tract infection	22.7%	10	30.8%	60	6.9%	2	Urinary tract infection	9.1%	4	11.3%	22	0.0%	0
Back pain	9.1%	4	20.0%	39	3.4%	1	Contusion	6.8%	3	11.3%	22	0.0%	0

Japanese clinical studies (study duration, up to 38 weeks): C07-001 and E07-001 (Interim report)
Foreign clinical studies (study duration, up to 206 weeks): TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, X03-001A, and E05-001 (Interim report)

Table 43. Serious adverse events occurring in at least 2 patients in either group during the entire treatment period in clinical studies (Pooled analysis)

	Foreign clinical studies				Japanese clinical studies			Foreign clinical studies				Japanese clinical studies	
	Placebo (N = 44)		Eculizumab (N = 195)		Eculizumab (N = 29)			Placebo (N = 44)		Eculizumab (N = 195)		Eculizumab (N = 29)	
	Incidence	N	Incidence	N	Incidence	N		Incidence	N	Incidence	N	Incidence	N
Overall	20.5%	9	25.6%	50	13.8%	4	Headache	0.0%	0	2.1%	4	0.0%	0
Abdominal pain	0.0%	0	1.0%	2	0.0%	0	Convulsion	0.0%	0	1.0%	2	0.0%	0
Pyrexia	2.3%	1	3.6%	7	3.4%	1	Acute renal failure	0.0%	0	1.0%	2	3.4%	1
Cholelithiasis	0.0%	0	1.0%	2	0.0%	0	Nephrolithiasis	0.0%	0	1.0%	2	0.0%	0
Cellulitis	2.3%	1	1.0%	2	3.4%	1	PNH	6.8%	3	1.0%	2	0.0%	0
Viral infection	2.3%	1	2.6%	5	0.0%	0	Anaemia	2.3%	1	2.1%	4	3.4%	1
Viral gastroenteritis	0.0%	0	1.0%	2	0.0%	0	Haemolytic anaemia	0.0%	0	1.0%	2	0.0%	0
Septic shock	0.0%	0	1.0%	2	0.0%	0	Thrombocytopenia	0.0%	0	1.0%	2	0.0%	0
Intervertebral disc protrusion	0.0%	0	1.5%	3	0.0%	0	Neutropenia	4.5%	2	0.5%	1	0.0%	0

Japanese clinical studies (study duration, up to 38 weeks): C07-001 and E07-001 (Interim report)
Foreign clinical studies (study duration, up to 206 weeks): TRIUMPH, SHEPHERD, C02-001, E02-001, X03-001, X03-001A, and E05-001 (Interim report)

(6) Indication

PMDA concluded as follows:

Eculizumab is palliative and is not a curative therapy for PNH. Improvement in survival prognosis has not been demonstrated at present. Eculizumab may increase the risk of developing an infection with encapsulated bacteria, e.g. *Neisseria meningitidis*. Once treatment is initiated, continued treatment is needed. Taking account of these points, eculizumab should be used only in appropriate patients, fully examining treatment effects and potential risks. Therefore, the package insert should state that Hb stabilization and reductions in transfusion requirements have been demonstrated only in the TRIUMPH study population and advise that eculizumab should be used only if the clinical benefits are considered to outweigh the potential risks. It is also necessary to state that the efficacy of eculizumab during an acute hemolytic crisis has not been demonstrated.

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The expert advisors made the following comments and supported the above conclusions by PMDA.

- PNH is considered associated with a relatively good survival prognosis, in view of the age of onset and survival time after diagnosis (Table 19). As the effects of eculizumab on the development of TE and survival prognosis are undefined, the significance of administering eculizumab to less transfusion-dependent patients is not high and it should be advised that eculizumab should be used in patients who are expected to require regular transfusions.
- Eculizumab reduces hemolysis, but may increase the risk of a serious hemolytic crisis due to the accumulation of PNH type III RBCs.
- Prior to the use of eculizumab, risk-benefit assessment is needed on an individual basis and it is difficult to define the intended population, but appropriate patients may be selected based on the patient populations enrolled in clinical studies.
- Given the risks associated with eculizumab, adequate information should be provided to ensure that eculizumab will not be initiated easily in patients in less need of treatment with eculizumab.
- In clinical practice, it is envisaged that treatment initiation will be considered if a patient is hospitalized due to an acute hemolytic crisis, etc. Thus, it should be stated that the efficacy of eculizumab in such cases is unknown.

Based on the above comments from the expert advisors, PMDA instructed the applicant to modify the proposed indication and precautions of indication statements of the package insert. The applicant responded that the statements will be modified as follows and PMDA accepted it.

[Indication]

Reduction of hemolysis in patients with paroxysmal nocturnal hemoglobinuria

[Precautions for indication]

- Treatment with Soliris should be initiated in patients with a diagnosis of paroxysmal nocturnal hemoglobinuria established by flow cytometry etc.
- Treatment with Soliris should be initiated in patients considered to require RBC transfusions due to hemolysis and expected to require regular transfusions.
- Prevention of thromboembolism, improvement in renal function, or survival benefit of Soliris has not been demonstrated.
- The efficacy of Soliris during an acute hemolytic crisis has not been demonstrated.
- Due to the following potential risks, after fully understanding the efficacy and safety of Soliris, whether or not to use Soliris should be determined carefully and treatment with Soliris should be initiated in appropriate patients (see “Clinical Studies”).
  - ► An increased risk of infections with *Neisseria meningitidis* etc. (Soliris inhibits complement C5 and may increase a patient’s susceptibility to infections with encapsulated bacteria, e.g. *Neisseria meningitidis*.)

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► Development of a serious hemolytic crisis after Soliris discontinuation (As PNH RBCs increase in patients being treated with Soliris, serious intravascular hemolysis may be induced if Soliris is discontinued.)

(7) Dosage and administration

PMDA concluded as follows:

Although the rationale for the proposed dosage and administration is not necessarily sufficient, the TRIUMPH study has confirmed the efficacy of eculizumab, Study C07-001 in Japanese PNH patients also has suggested reduction in hemolysis, and based on the submitted clinical study data, eculizumab increases the risk of developing a meningococcal infection, but otherwise the safety profile of eculizumab is tolerable. Therefore, eculizumab has certain usefulness at the dosage regimen used in clinical studies in PNH patients. It should be stated in the precautions for dosage and administration section of the package insert that as a hemolytic crisis due to decreased blood concentrations may occur, the labeled dosage and administration instructions should be complied with and that if LDH levels are not reduced for a certain period of time, whether or not to continue treatment should be determined.

The above conclusions by PMDA were supported by the expert advisors and PMDA instructed the applicant to modify the dosage and administration and precautions for dosage and administration statements of the package insert.

The applicant explained that although LDH levels were reduced by Week 1 in the majority of patients, as it took about 2 weeks for LDH levels to be reduced in some patients, whether or not to continue treatment with eculizumab should be determined at around Week 2. The applicant then modified the dosage and administration and precautions for dosage and administration statements as follows and PMDA accepted it.

[Dosage and administration]

The usual adult dosage is 600 mg as Eculizumab (Genetical Recombination) given as an intravenous infusion weekly for a total of 4 doses, followed 1 week later (4 weeks after the first dose) by 900 mg given every 2 weeks thereafter.

[Precautions for dosage and administration]

- Diluted Soliris should be administered by intravenous infusion over 25 to 45 minutes via a dedicated infusion line.
- Comply with the recommended dosing intervals as an acute hemolytic crisis due to decreased blood concentrations of Soliris may occur.
- If serum lactate dehydrogenase (LDH) activity is not reduced by Week 2, whether or not to continue treatment with Soliris should be determined.

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(8) Pediatric indication

In response to the questions on a pediatric indication, the applicant explained as follows:

Among 34 pediatric patients treated with eculizumab in the foreign marketing experience (as of August 24, 2009), 6 patients experienced serious adverse events ("central line infection" in 1 patient, "anaemia and *Pseudomonas aeruginosa* infection" in 1 patient, "tooth infection" in 1 patient, "thrombosis, abdominal pain, and constipation" in 1 patient, "bronchospasm" in 1 patient, "respiratory failure, cerebral aspergillosis, and enterococcal sepsis" in 1 patient). A causal relationship to eculizumab could not be denied for "anaemia and *Pseudomonas aeruginosa* infection" and no causality assessment was reported for "tooth infection," "thrombosis, abdominal pain, and constipation," and "bronchospasm."

Although there is no plan to develop eculizumab for Japanese pediatric patients with PNH at present, the development of eculizumab for pediatric use will be considered based on the planned foreign clinical study and the uses of eculizumab in Japan.

PMDA concluded as follows:

As it is known that children also develop PNH, a pediatric indication should also be considered. Due to very limited information on the efficacy and safety of eculizumab in children at present, for the time being, the phrase "adult dosage" should be included in the dosage and administration statement and it should be stated in the precautions section that "the efficacy and safety of eculizumab in children has not been established."

The following comment on the above conclusions by PMDA was raised from the expert advisors: as the risk of infections is different between adults and children, special caution is required when using eculizumab in children. The conclusions by PMDA were supported by the expert advisors.

If a pediatric patient treated with eculizumab is reported from a post-marketing surveillance study covering all treated patients, the case should be reviewed and it will be necessary to provide information and take safety measures as appropriate [see "(9) Post-marketing surveillance etc."].

(9) Post-marketing surveillance etc.

In response to the request from PMDA, the applicant explained an overview of the PNH registry, a foreign epidemiological study, as follows:

The objectives of the study are to capture the clinical courses of patients with PNH and to raise awareness of PNH among healthcare professionals and patients. In the study, patient demographics, the drugs used, disease progression, and clinical outcomes have been surveyed based on the data collected during patient visits.

The applicant explained that based on this PNH registry, a post-marketing survey (survey period, 8

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years; a survey covering all treated patients) as shown in Table 38 will be conducted in Japan.

PMDA concluded that in addition to the items presented by the applicant, (a) PNH type III RBC clone over time (collect the information if analyzed), (b) the information on HAHA (antibody titers, neutralizing antibodies, etc.), and (c) the information on the use of eculizumab in children and pregnant women/nursing mothers should be collected. This conclusion was supported by the expert advisors. The expert advisors commented that the following items should also be included: (d) the reason for definitive diagnosis of PNH, (e) concurrent AA or MDS, (f) the information on the cases of lack of efficacy, (g) the information on the use of eculizumab during an acute hemolytic crisis, (h) the information on meningococcal infections (meningococcal carriage [if tested], a history of meningococcal infection, meningococcal vaccination status [vaccination date, vaccine type, etc.]), (i) detailed information on the development of infections including meningococcal infection, (j) the development of TE during treatment period, and (k) the reason for eculizumab discontinuation and the severity of hemolysis after discontinuation.

PMDA instructed the applicant to submit a draft outline of a post-marketing surveillance plan incorporating the above points and the applicant submitted an outline of a post-marketing surveillance plan (draft) as summarized in Table 44 and PMDA accepted it.

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Table 44. Outline of post-marketing surveillance plan (draft)

Objectives	・Detection of unknown and serious adverse drug reactions ・Long-term safety and efficacy ・Effects of eculizumab on the course of PNH (progression, course, and prognosis etc. of the disease)
Survey period	Re-examination period
Planned number of patients	All patients treated with eculizumab
Target patient population	Patients diagnosed with PNH
Major items to be investigated	(a) Patient background analysis: Indication for use: reduction of hemolysis in patients with paroxysmal nocturnal hemoglobinuria ・The reason for definitive diagnosis of PNH (the basis of the diagnosis) Priority items (Patient background for safety) ・Meningococcal carriage (if tested) Risk analysis of healthy carriers and noncarriers, Effects of eculizumab ・History of meningococcal infection Risk analysis of patients with or without a history of meningococcal infection, Effects of eculizumab ・Meningococcal vaccination (vaccination date, vaccine type) Risk analysis by vaccination status, vaccine type, and time after vaccination, Effects of eculizumab (b) Priority items (Safety) ・Development of infections during treatment period (especially, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*) Risk analysis of the development of infections, Effects of eculizumab ・Development of thromboembolism during treatment period Risk analysis of the development of thromboembolism, Effectiveness of eculizumab ・Presence or absence of concomitant medications for the prevention of thromboembolism Risk analysis of the development of thromboembolism, Effectiveness of concomitant medications ・Development and severity of hemolysis after eculizumab discontinuation, the reason for discontinuation Safety analysis after the withdrawal of eculizumab ・Development of headache symptoms (including associated symptoms), course, outcome, cause (meningococcal infection, other infections, infusion reactions to eculizumab, etc.), treatment instituted (c) Other items Information required for safety factor analysis will be focused, but efficacy items will also be investigated. ・Concomitant medications: Effects of corticosteroids, immunosuppressants, erythropoietin, and anticoagulants on the safety and efficacy of eculizumab ・Other medical history etc. (already cured/remitted before the initiation of treatment): Effects of eculizumab on the development (recurrence) of major vascular events and other diseases ・Underlying disease (concurrent disease) etc.: Effects of AA, MDS, or other myeloid disorders on the safety and efficacy of eculizumab ・PNH duration etc., Time after PNH diagnosis: Effects of time elapsed after definitive diagnosis on the effectiveness of eculizumab ・Complications (comorbidities/symptoms) etc.: Effects of major vascular events, hepatic impairment/renal impairment, or the symptoms of other complications/comorbidities etc. on the safety and efficacy of eculizumab ・Change in LDH from baseline over time ・Improvement in QOL (fatigue, abdominal pain, dyspnoea, dysphagia) ・Change in Hb from baseline over time ・Change in the number of PRBC units transfused from baseline over time ・Change from baseline in PNH type III or II RBCs (if analyzed) ・Change from baseline in the percentage of PNH type I, II, or III RBCs as measured by flow cytometry (if analyzed) ・Cases of lack of efficacy (Identify its cause) ・Efficacy of eculizumab during an acute hemolytic crisis ・Information on HAHA (antibody titers, neutralizing antibodies, etc.) (if assay is requested by the medical institution)

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III. Overall Evaluation

As a result of the above review, PMDA concludes that eculizumab may be approved after modifying the indication and dosage and administration statements as shown below, with the following conditions. As eculizumab is an orphan drug, the re-examination period is 10 years, the drug substance and the drug product are both classified as powerful drugs, and the product is classified as a biological product.

[Indication]

Reduction of hemolysis in patients with paroxysmal nocturnal hemoglobinuria

[Dosage and administration]

The usual adult dosage is 600 mg as Eculizumab (Genetical Recombination) given as an intravenous infusion weekly for a total of 4 doses, followed 1 week later (4 weeks after the first dose) by 900 mg given every 2 weeks thereafter.

[Conditions for approval]

1. Due to limited data from Japanese clinical studies, conduct a post-marketing drug use-results survey over a certain period of time, covering all patients treated with Soliris, in order to obtain the background information of patients treated with Soliris, collect data on the safety and efficacy of Soliris, and take necessary measures to ensure proper use of Soliris.
2. Prior to marketing Soliris, take necessary measures to ensure that Soliris will be administered only under the supervision of a physician at a medical institution, who is familiar with the diagnosis and treatment of paroxysmal nocturnal hemoglobinuria and is also fully capable of managing the risks etc. associated with Soliris in cooperation with a physician who is familiar with the diagnosis and treatment of meningococcal infection.

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Page 958

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15260888	BELL ET AL.
	Examiner	Art Unit
	JAMES ROGERS	1644

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
STN: DISSABS MEETINGS BIOSCI CAPLUS MEDICINE	10/19/2016	JR
EAST: PGPUB, USPAT, JPO, EPO, INVENTOR SEARCH	10/19/2016	JR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
	USPAT, USPAD, SEQ ID NO: 2, 4	10/19/2016	JR

/JAMES ROGERS/ Examiner.Art Unit 1644	

Doc code: IDS
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	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

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	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

	1	THOMAS et al., Inhibition of complement activity by humanized anti-C5 antibody and single; chain Fv, Mol. Imm. 33 (17-18):1389-1401 (1996);	
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EXAMINER SIGNATURE

Examiner Signature	/JAMES L ROGERS/	Date Considered	10/24/2016

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	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

CERTIFICATION STATEMENT

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

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

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A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Jill Gorny Sloper/	Date (YYYY-MM-DD)	2016-10-21
Name/Print	Jill Gorny Sloper, Esq.	Registration Number	60,760

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SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/260,888	09/09/2016	424	1644	AXJ-114BUSCN3

APPLICANTS

Alexion Pharmaceuticals, Inc., New Haven, CT;

INVENTORS

Leonard BELL, Woodbridge, CT;
Russell P. Rother, Oklahoma City, OK;

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

This application is a CON of 15/148,839 05/06/2016
which is a CON of 13/426,973 03/22/2012
which is a CON of 12/225,040 05/13/2009 ABN
which is a 371 of PCT/US2007/006606 03/15/2007

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Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☑ No Verified and Acknowledged /JAMES L ROGERS/ Examiner's Signature	☐ Met after Allowance JR Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
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ADDRESS

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

TITLE

TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

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	32	Osbourn, J. et al., "Current methods for the generation of human antibodies for the treatment of autoimmune diseases," Drug Discovery Today, vol. 8 (18): 845-851 (2003)	
	33	Other News To Note, BIOWORLD Today (Aug 9, 2002): 5 pages.	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

	34	PARKER et al., Diagnosis and management of paroxysmal nocturnal hemoglobinuria, Blood; 106(12):3699-3709 (2005);	
	35	Paroxysmal nocturnal hemoglobinuria, included; PNH, included phosphatidylinositol glycan,; class A, pseudogene 1, included; pigap1, included pig a-related processed gene, included; [online]. OM 1M-Online Mendelian Inheritance in Man; John Hopkins University; [Retrieved on; 2003-06-18]. Retrieved from the Internet:; http://www.ncbi.nlm.nih.gov/entrezlquery.fcgi?cmd=Retrieve&db=OMIM&dopt=detailed ...;	
	36	Pierangeli,S. et al., "Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombophilia," Arthritis & Rheumatology, vol. 52, Issue 7: 2120-2124(2005)	
	37	REITER et al., Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease,; Nature Med. 8 (12):1383-1389(2002);	
	38	RICHARDS et al., Evolution of GPI-Deficient Clones Predicts Clinical Course in Paroxysmal; Nocturnal Haemoglobinuria, Blood 104:Abstract 172 (2004);	
	39	Ricklin, D. et al., "Complement-targeted therapeutics," Nature Biotechnology, vol. 25(11): 1265-1275 (2007)	
	40	RODARY et al., "Patient preference for either the EORTC QLQ-C30 or the FACIT Quality of Life; (QOL) measures: a study performed in patient suffering from carcinoma of an unknown; primary site (CUP)," European Journal of Cancer, Vol. 40, pp. 521-528 (2004).	
	41	ROSSE et al., Immune-mediated hemolytic anemia, Hematology Am Soc Hematol Educ; Program, 48-62 (2004);	
	42	ROTHER et al., Eculizumab, a C5 complement-blocking antibody, abolishes hemolysis and; reduces transfusion dependency in patients with paroxysmal nocturnal hemoglobinuria (PNH),; Molecular Immunology, Vol. 40(2-4), page 197; XP009163498, (2003).;	
	43	ROTHER et al., The clinical sequelae of intravascular hemolysis and extracellular plasma; hemoglobin, JAM A 293 (13):1653-1662 (2005);	
	44	ROTHER et al.. "Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria," Nature Biotechnology, 25 (11) 1256-1264 (2007)	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

	45	SIERRA, G. et al., "Vaccine against group B Neisseia meningitidis: Protection trial and mass vaccination results in Cuba," NIPH ANNALS 1991 NO, vol. 14(2), pages 195-210, ISSN: 0332-5652 (1991)	
	46	STEBLER et al., High-dose recombinant human erythropoietin for treatment of anemia in; myelodysplastic syndromes and paroxysmal nocturnal hemoglobinuria: a pilot study, Exp.; Hematol. 18: 1204-1208 (1990);	
	47	STRAUSS et al. "Alternative pathway of complement in sickle cell disease" Pediat. Res. 11, 285-289 (1997)	
	48	STUART et al. "Sickle-cell disease" Lancet, 364:1343-1360 (2004)	
	49	TANG et al., "Design of Freeze-Drying Process for Pharmaceuticals: Practical Advice" Pharmaceutical Research, vol. 21 (2):191-200 (2004)	
	50	The MERCK MANUAL of Diagnosis and Therapy, Seventeenth Edition, Chapter 127 Anemias,; pp. 849-883 (1999).;	

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

EXAMINER SIGNATURE

Examiner Signature	/JAMES L ROGERS/	Date Considered	10/24/2016

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

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

CERTIFICATION STATEMENT

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

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

OR

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

See attached certification statement.

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

A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Jill Gorny Sloper/	Date (YYYY-MM-DD)	2016-10-21
Name/Print	Jill Gorny Sloper, Esq.	Registration Number	60,760

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

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

Privacy Act Statement

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

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

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

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

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

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

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

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

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

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

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

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

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: October 21, 2016
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, /

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888

Confirmation No.: 2558

Filed: September 9, 2016

Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT (IDS)

Dear Sir:

In compliance with 37 C.F.R. § 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the documents listed on the attached PTO/SB/08. It is respectfully requested that the documents listed on the PTO/SB/08 be expressly considered by the Examiner during the prosecution of this application, and that the documents be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

The present application is a continuation of U.S. Application No. 15/148,839, filed May 6, 2016 (Atty. Docket No. AXJ-114BUSCN2). For the Examiner's convenience in reviewing this continuation application, Applicant submits a consolidated PTO Form SB/08, listing all references cited during the prosecution of the parent applications [U.S. Application No. 15/148,839, filed May 6, 2016 (Atty. Docket No. AXJ-114BUSCN2), U.S. Application No. 13/426,973, filed March 22, 2012 (Atty. Docket No. AXJ-114BUSCN), and U.S. Application No. 12/225,040, filed May 13, 2009 (Atty. Docket No. AXJ-0114BUS)].

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

Copies of the references previously cited by or submitted to the Office in the parent applications are not enclosed, but will be provided upon request, in accordance with 37 CFR §1.98(d). In addition, Applicant submits herewith copies of foreign patents and non-patent literature references, but has not included copies of U.S. patents and U.S. patent applications, in accordance with 37 C.F.R. § 1.98(a)(2)(i)-(iv).

Additionally, Applicant calls to the attention of the Examiner the following Applications and Office Actions cited therein:

Applications				
Examiner's Initials	*Serial No.*	*Filing Date*	*First Named Inventor*	*Docket No.*
	15/284,015	10-03-2016	Leonard Bell	AXJ-114BUSCN4
	15/148,839	05-06-2016	Leonard Bell	AXJ-114BUSCN2
	13/426,973	03-22-2012	Leonard Bell	AXJ-114BUSCN
	12/225,040	05-13-2009	Leonard Bell	AXJ-114BUS
	12/621,792	11-19-2009	Leonard Bell	AXJ-114DV
	10/771,552	02-03-2004	Leonard Bell	AXJ-114RCE
	11/050,543	02-03-2005	Leonard Bell	AXJ-114CPRCE
	11/595,118	11-08-2006	Leonard Bell	AXJ-114CP2
	12/902,617	10-12-2010	Leonard Bell	AXJ-114CPCN
	13/335,518	12-22-2011	Leonard Bell	AXJ-114CPCN2RCE

Office Actions (copies enclosed)			
Examiner's Initials	Serial No.	Date Mailed from USPTO	Examiner
	15/148,839	08-16-2016	J. Rogers
	13/426,973	01-27-2014	J. Rogers
	13/426,973	05-31-2013	J. Rogers
	13/426,973	08-20-2012	J. Rogers
	12/225,040	09-22-2011	J. Rogers
	12/225,040	06-01-2011	J. Rogers
	12/225,040	04-11-2011	J. Rogers
	12/621,792	09-30-2010	P. Gambel
	10/771,552	12-22-2008	F. Vandervegt
	10/771,552	03-21-2008	F. Vandervegt
	10/771,552	08-10-2007	F. Vandervegt
	10/771,552	02-26-2007	F. Vandervegt
	10/771,552	09-26-2006	F. Vandervegt

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

	11/050,543	07-09-2010	P. Gambel
	11/050,543	04-02-2008	F. Vandervegt
	11/050,543	10-10-2007	F. Vandervegt
	11/050,543	06-11-2007	F. Vandervegt
	11/595,118	01-21-2011	P. Gambel
	11/595,118	10-05-2010	P. Gambel
	11/595,118	07-07-2010	P. Gambel
	11/595,118	04-03-2009	F. Vandervegt
	12/902,617	06-23-2011	P. Gambel
	12/902,617	04-04-2011	P. Gambel
	13/335,518	08-22-2016	P. Gambel
	13/335,518	09-12-2014	P. Gambel
	13/335,518	02-06-2014	P. Gambel
	13/335,518	11-07-2013	P. Gambel

/JAMES L ROGERS/ 10/24/2016

The Examiner is requested to review the file histories of these applications, including cited references, Office Actions, Responses, etc., and is asked to contact Applicant's Attorney if the Examiner would like the Applicant to supply copies of any or all of the information included in any of these applications. For any of these applications, if Applicant's Attorney is not contacted by the Examiner with such a request, then it will be concluded that the Examiner has reviewed or will review the file content of these applications.

Applicant respectfully requests that the Examiner initial the blank columns next to the cited Applications and Office Actions, to indicate that the information has been considered by the Examiner. Alternatively, Applicant requests that the Examiner insert the phrase, "All references considered except where lined through," on each page of the Information Disclosure Statement, along with the Examiner's initials.

The filing of this Information Disclosure Statement is not to be interpreted as a representation that the cited documents are material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any documents herein be construed *per se* as a representation that such document is prior art. Moreover, Applicant understands the Examiner will make an independent evaluation of the cited documents.

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

This Information Disclosure Statement is filed within three months of the U.S. filing date (37 C.F.R. § 1.97(b)(1)). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3.

Dated: October 21, 2016

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, /
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

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

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
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The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

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Application	Document	Mailroom Date	Attorney Docket No.
15260888	CTNF	10/27/2016	AXJ-114BUSCN3
	892	10/27/2016	AXJ-114BUSCN3
	1449	10/27/2016	AXJ-114BUSCN3
	1449	10/27/2016	AXJ-114BUSCN3
	1449	10/27/2016	AXJ-114BUSCN3

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If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: November 4, 2016
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888 Confirmation No.: 2558

Filed: September 9, 2016 Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT IN RESPONSE TO

NON-FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.111

Dear Sir:

In response to the Office Action dated October 27, 2016 (Paper No. 20161019), please amend the above-identified U.S. patent application as follows:

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

A Listing of the Claims begins on page 3 of this paper.

Remarks/Arguments begin on page 5 of this paper.

AMENDMENTS TO THE SPECIFICATION

In the Sequence Listing:

Please replace the Sequence Listing filed on September 9, 2016 with the replacement Sequence Listing submitted herewith.

In the Specification:

Please replace the "Sequence Listing" Paragraph on page 1 of the specification with the following paragraph:

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on ~~September 8, 2016~~ November 3, 2016, is named AXJ_114BUSCN3_Seq_Listing.txt and is ~~10,483~~ 10,282 bytes in size.

Please amend the paragraph beginning on page 44 (lines 24-29) as follows:

SEQ ID NO: 4 – Eculizumab Light Chain
~~MDMRVPAQLLGLLLLWLRGARC~~DIQMTQSPSSLSASVGDRVTITCGASENIYGAL
NWYQQKPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
NVLNTPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC

LISTING OF THE CLAIMS

1. **(Original)** A method of treating a patient suffering from paroxysmal nocturnal hemoglobinuria (PNH) comprising administering to the patient a pharmaceutical composition comprising an antibody that binds C5, wherein the antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

2. **(Original)** The method of claim 1, wherein the pharmaceutical composition is administered by intravenous infusion.

3. **(Original)** The method of claim 1, wherein the antibody is administered to the patient at a dosage level of between 5 mg per kg and 50 mg per kg per patient per treatment.

4. **(Original)** The method of claim 1, wherein the pharmaceutical composition is in a single unit dosage form.

5. **(Original)** The method of claim 4, wherein the single unit dosage form is a 300 mg single unit dosage form.

6. **(Original)** The method of claim 1, wherein the pharmaceutical composition comprises a 300 mg single-use formulation of 30 ml of a 10 mg/ml sterile, preservative free solution.

7. **(Original)** The method of claim 1, wherein the patient is anemic.

8. **(Original)** The method of claim 1, wherein the patient is dosed as follows: 600 mg of the antibody via intravenous infusion every 7±1 days for 4 doses; followed by 900 mg of the antibody via intravenous infusion 7±1 days later; followed by a maintenance dose of 900 mg of the antibody via intravenous infusion every 14±2 days.

9. **(Original)** The method of claim 1, wherein administration of the antibody results in an immediate and sustained decrease in mean levels of lactate dehydrogenase (LDH).

10. **(Original)** The method of claim 9, wherein the immediate decrease occurs within one week of administration of the antibody.

REMARKS

Applicants acknowledge with appreciation the telephonic interview with the Examiner and his Supervisor on November 1, 2017, during which the rejections were discussed, as well as the fact that eculizumab was not in public use or publicly available as of March 15, 2007.

Claims 1-10 are pending. Applicants reserve the right to pursue claims to any canceled subject matter, or any subject matter which they are entitled to claim, in this or a separate application. *No new matter has been added.*

Information Disclosure Statement

Applicants acknowledge with appreciation the Examiner's indication that the Information Disclosure Statement submitted on October 21, 2016 has been considered. However, only three out of the four PTO/SB/08a Forms were formally acknowledged. Accordingly, Applicants respectfully request that the Examiner provide an initialed copy of the fourth PTO/SB/08a that was filed on October 21, 2016, to indicate that the listed references have been considered.

Additionally, Applicants submit herewith a Supplemental Information Disclosure Statement to make of record additional information for the Examiner's consideration.

Obviousness-Type Double Patenting

The Examiner has provisionally rejected claims 1-10 on the ground of non-statutory obviousness-type double patenting as being unpatentable over claim 1 of co-pending U.S. Patent Application No. 15/148,839.

While in no way acquiescing to the rejection, Applicants respectfully submit herewith a terminal disclaimer over U.S. Patent Application No. 15/148,839, as well as U.S. Patent Application No. 15/284,015. Accordingly, Applicants respectfully request that the Examiner withdraw the rejection.

Replacement Sequence Listing and Amendment to the Specification

It has come to Applicants' attention that the light chain recited in the specification and Sequence Listing as SEQ ID NO:4 inadvertently contains the precursor peptide leader sequence, as shown below:

SEQ ID NO: 4 (Light Chain)
MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCGASE
NIYGALNWYQQKPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQNVLNTPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQL
KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Accordingly, Applicants submit herewith a replacement Sequence Listing amending SEQ ID NO:4 to delete the leader sequence (underlined above), which is ultimately cleaved from the active light chain. Thus, as amended, SEQ ID NO:4 corresponds to the mature portion of the complete light chain sequence of eculizumab. Applicants have also amended the specification to update the "Sequences" summary on page 44 (lines 24-29) of the specification.

Applicants respectfully note that the mature light chain sequence is an inherent portion of the precursor sequence (*i.e.*, original SEQ ID NO:4), and could have been readily identified at the relevant filing date using well established rules and art-recognized techniques (see, for example, Figure 6 on page 734 of Adderson *et al.*, *J. Clin. Invest.* 89:729-738 (1992) (enclosed herewith as Exhibit A), which shows the characteristic mature N-terminus (DIQ) of a light V kappa antibody light chain, as well as Kabat *et al.*, Sequences of Immunological Interest 5th Edition, U.S. Public Health Service, Bethesda MD). Therefore, the amendment to SEQ ID NO:4 does not constitute new matter.

CONCLUSION

In view of the foregoing, entry of the amendments and remarks herein, reconsideration and withdrawal of all rejections, and allowance of the instant application with all pending claims is respectfully solicited. If a telephone conversation with the Applicants` attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call the Applicants` attorney at (617) 217-4630.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3.

Dated: November 4, 2016

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

EXHIBIT A:
Adderson *et al*., *J. Clin. Invest*. 89:729-738 (1992)

Immunoglobulin Light Chain Variable Region Gene Sequences for Human Antibodies to *Haemophilus influenzae* Type b Capsular Polysaccharide Are Dominated by a Limited Number of V_κ and V_λ Segments and VJ Combinations

Elisabeth E. Adderson,* Penelope G. Shackelford,* Richard A. Insel,§ Anthony Quinn,* Patricia M. Wilson,‡ and William L. Carroll‖

*Division of Infectious Diseases and ‡Hematology Oncology, Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis Children's Hospital, St. Louis, Missouri 63110; and §Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

Abstract

The immune repertoire to *Haemophilus influenzae* type b capsular polysaccharide (Hib PS) appears to be dominated by certain light chain variable region genes (IgV_L). In order to examine the molecular basis underlying light chain bias, IgV_L genes have been cloned from a panel of heterohybridomas secreting human anti-Hib PS (antibody) (anti-Hib PS Ab). One hybridoma, representative of the predominant serum clonotype of anti-Hib PS Ab in older children and adults following immunization or Hib infection, uses a V_κII segment identical to the germline gene A2, and a JK3 segment. A second kappa hybridoma uses a member of the V_κI family and a JK4 segment. Four lambda antibodies, all cross-reactive with the structurally related antigen *Escherichia coli* K100 PS, use V_λVII segments which are 96–98% homologous to one another, and may originate from a single germline gene. Two additional lambda antibodies, not K100-cross-reactive, are encoded by members of the V_λII family. All lambda antibodies use highly homologous J_λ2 or J_λ3 segments. The VJ joints of all lambda antibodies and the V_κII-encoded antibody are notable for the presence of an arginine codon, suggesting an important role in antigen binding. Although more complex than heavy chain variable region gene usage, a significant portion of serum anti-Hib PS Ab is likely to be encoded by a limited number of V_κ and V_λ segments and VJ combinations, which may be selectively expressed during development, or following antigen exposure. (*J. Clin. Invest.* 1992: 89:729–738.) Key words: B-cell repertoire • somatic mutation • gene rearrangement • carbohydrate antigen

Introduction

The development of the antibody repertoire involves the recombination of individual members of two or three groups of germline segments, nucleotide insertion or deletion at these junctions, and somatic mutation of rearranged genes (1–4). The individual contributions of these molecular mechanisms to the generation of the immune repertoire against a specific antigen has been extensively studied in murine systems, but it is uncertain whether these findings can be extrapolated to humans. The chromosomal organization of the human heavy and light chain variable region segments within the immunoglobulin loci differ from those of mice (5–7). Furthermore, the response of highly inbred laboratory animal strains, and the use of simple haptens in most studies, may not be comparable with the human response to clinically relevant antigens, such as those presented by microbial pathogens.

The immune response to *Haemophilus influenzae* type b capsular polysaccharide (Hib PS)[1] is an excellent model to examine the immune repertoire to a functionally significant antigen. Hib is a major bacterial pathogen in young children and antibody directed against the capsular polysaccharide, a repetitive polymer of polyribosylribitol phosphate, protects against invasive infection (8). Similar to the response to other polysaccharide antigens, the immune response to Hib PS is age-dependent. Children under 5 yr have lower antibody responses and are at greatest risk of infection. Additionally, there appear to be variations in individual and ethnic susceptibilities to invasive Hib disease (9–11). Analysis of the immune response to Hib PS will contribute to our understanding of the development of the human antibody repertoire, and provide insight into age-related and possible genetic predisposition to infection caused by Hib.

Previous studies employing partial amino acid sequencing of anti-Hib PS serum antibody purified to clonality have shown that light chains of anti-Hib PS antibody are encoded by V_λ, and at least three V_κ gene families (12). Although highly informative, these studies have inherent technologic limitations. To obtain sufficient antibody for analysis, only individuals with antibody concentrations much higher than those elicited in young infants could be studied (12). Amino acid sequences were unobtainable on V_λ-encoded antibodies, and certain regions of V_κ-encoded antibodies (12, 13). Obtaining nucleic acid sequences of variable region genes encoding anti-Hib PS Abs would permit precise comparison of variable region genes obtained from different individuals, allowing classification of these genes into germline families and subfamilies, analysis of D- and J-segment usage, and assessment of the role of somatic mutation in the clonal evolution of this immune response. Additionally, this information could be applied to examination of the germline and expressed immune repertoire

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Address correspondence and reprint requests to Dr. William L. Carroll, Program in Human Molecular Biology and Genetics, University of Utah, Bldg. 533, Salt Lake City, UT 84112.

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1. *Abbreviations used in this paper:* Ars, anti-p-azophenylarsonate; Hib, Haemophilus influenzae type b; Hib PS, Hib capsular polysaccharide; IgV_L, light chain variable region gene; PCR, polymerase chain reaction; SSC, standard saline citrate.

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of individuals of various ages, and populations at high risk for invasive Hib infection.

Our previous work, and that of others, indicated that the heavy chains of anti-Hib PS Abs are encoded by a very restricted number of V_HIII gene segments, in combination with a variety of D and J_H segments (12, 14). We have extended our studies to examine light chain variable region gene (IgV_L) usage in the human anti-Hib PS response. A major component of serum anti-Hib PS Ab expresses a cross-reactive idiotype associated with the use of a V_κII gene (15, 16). Antibodies expressing this light chain account for the majority of anti-Hib PS antibody in older infants and adults following invasive Hib disease or immunization. Most lambda-encoded anti-Hib antibodies and some kappa-encoded (V_κI and V_κIII) anti-Hib PS Abs cross-react with the capsular polysaccharide of *E. coli* K100 (12, 17). Naturally acquired antibody of the majority of children expresses K100 PS cross-reactivity, and thus, colonization with this organism may provide protective immunity before exposure to Hib (18). Our results indicate that two important anti-Hib PS light chains, one associated with V_κII expression and the other with lambda antibodies cross-reactive with K100 PS, are encoded by two germline elements that may be expressed with little or no somatic mutation. Moreover, a nongermline-encoded arginine at the VJ joint is seen in the majority of anti-Hib PS Ab light chains, a result suggesting an essential role in antigen recognition.

Methods

Hybridomas. Heterohybridomas secreting human monoclonal anti-Hib PS antibody were obtained, as previously described (14). Volunteers were immunized with either plain Hib-PS vaccine (Praxis Biologicals, Rochester, NY) or Hib-PS diphtheria toxoid conjugate vaccine (Hib-PS-D) (Connaught Laboratories, Swiftwater, PA). 7 d following immunization, peripheral blood lymphocytes were harvested and fused to the nonsecreting mouse myeloma SP2/O-Ag14. Anti-Hib PS Ab was detected by binding to Hib PS in an ELISA, as previously described (14). Specificity was documented by antibody binding to ^{125}I-labeled antigen (19), and by inhibition of binding by 1.25 μg/ml of soluble Hib PS in an ELISA (20). Cross-reactivity with *E. coli* K100 PS was assayed by inhibition of binding to Hib PS-poly-L-lysine with 100 μg/ml of soluble K100 PS in an ELISA. An antibody concentration was selected that gave half-maximum absorbance in the ELISA. Greater than 15% inhibition was considered positive. The *E. coli* K100 PS was prepared from *E. coli* strain Easter, by the same method used to prepare Hib PS (19). The generation of hybridoma line 16M3C8 was described previously (21).

Cloning and sequencing of IgV_L genes. Total RNA was prepared from 10^6 hybridoma cells using the guanidine isothiocyanate method (22). 2 to 8 μg of total RNA served as template for first-strand cDNA synthesis, as previously described (14). 10 pmol of a consensus antisense primer corresponding to conserved sequences within the human kappa or lambda constant region (23) (C_κ codons 181-172:5′CAGCGTCAAGCTTCTGCTGTGGCTGTAGGT 3′, C_λ codons 171-181:5′CAGGCTCAGGAAGCTTCTGGCCGCGTACTTGTT 3′) was used to initiate cDNA synthesis by avian myeloblastosis virus (AMV) reverse transcriptase (RT; Life Sciences, St. Petersburg, FL). One half (10 μl) of the cDNA reaction was directly diluted into polymerase chain reactions (PCR), (24).

PCR conditions were as previously described, except that 70 pmol of an internal constant region consensus primer (C_κ codons 125-117:5′CAACGGATCCTCTGATGGCGGGAAGAT 3′, C_λ codons 125-117:5′TGGGGATCCAGCTCCTCAGAGGAGGG 3′) and 70 pmol of a degenerate 3′ kappa or lambda leader sequence primer (kappa:5′GGGAATTCATGGACATG(GA)(GA)(GA)(GAT)(TC)CC-(ACT)(GAC)G(TC)(GT)CA(GC)CTT 3′, lambda 5′GGGAATTCATG(GA)CCTG(GC)(AT)C(TC)CCTCTC(TC)T(TC)CT(GC)(AT)-(TC)C 3′) were used to amplify rearranged variable region genes (14, 25).

PCR products were isolated from low melting-point agarose gels (FMC Bioproducts, Rockland, ME). Artificial restriction enzymes sites in each primer allowed directional cloning into M13mp18 and M13mp19 phage vectors (Boehringer Mannheim Corp., Indianapolis, IN). Sequencing of V_L genes was performed by the dideoxy technique (26). Two to five positive clones were obtained and sequenced for each hybridoma gene.

The 16M3C8 light chain was generated by amplification of first-strand cDNA, with primers corresponding to amino acid positions −7 of the leader, to position 6 of the lambda light chain (5′CTGCACAGGGTCCTGGGCCGAGCTCGTGGTGACTCA 3′), and the C_λ constant region (antisense, 5′GCATTCTAGACTATTATGAACATTCTGTAGGGGC3′). Blunt-ended PCR products were cloned into SmaI cut M13 mp18 and M13mp19 and sequencing was also performed by the dideoxy method.

Southern blot analysis of genomic DNA. Peripheral blood was obtained from 10 unrelated healthy adult volunteers, from L.S. (the donor of hybridoma LSF2), and from a parent of L.S. Lymphocytes were separated on a Ficoll-Hypaque gradient, and genomic DNA was prepared by Proteinase K digestion (27). 10 μg of each subject's genomic DNA was digested with BamHI (4 U/μg DNA) (Boehringer Mannheim Corp.) at 37°C for 16 h, according to manufacturer's specifications. Digested DNA was separated by electrophoresis in a 0.8% agarose gel using TAE buffer (0.04 M Tris-acetate/0.001 M EDTA), and was transferred to Duralon UV membranes (Stratagene Inc., La Jolla, CA), using a positive pressure blotting apparatus (Posiblotter; Stratagene, Inc.). To examine germline representation of anti-Hib PS Ig genes, genomic DNA was hybridized with a 320-bp EcoRI/SmaI V_λ segment from hybridoma LSF2 that lacked the associated J_λ and C_λ regions. Probe was radiolabeled to high specific activity using T7 DNA polymerase (28). Prehybridization and hybridizations were carried out in 5× standard saline citrate (SSC), 5× Denhardt's solution, and 200 μg/ml sheared and denatured salmon sperm DNA (Boehringer Mannheim Corp.) at 42°C overnight. Filters were washed with 2× SSC, 0.2% SDS at room temperature twice, then with 0.2× SSC, 0.2% SDS at 55 to 60°C twice, and exposed to X-OMAT AR film (Eastman Kodak Co., Rochester, NY) for 2–7 d.

In order to further localize germline candidates within V region families, an oligonucleotide probe corresponding to conserved sequences within the CDR-1 region (sense oligonucleotide: 5′GGCTCCAGCACTGGAGATGTCACCAGT 3′, antisense oligonucleotide: 5′GTAGGGATAATGACCACTGGTGACATCTCC 3′) was hybridized to genomic DNA using the method described by Hellman (29). 15 μg of BamHI-digested human genomic DNA was separated by electrophoresis in 0.8% agarose gel using TBE buffer (0.09 M Tris-borate/0.002 M EDTA). Agarose gels were dried under vacuum and hybridized in 5× saline/sodium phosphate/EDTA (SSPE), 0.1% SDS, and 10^6 μg/ml of sheared and denatured salmon sperm DNA with 2×10^6 cpm of probe/ml of hybridization solution at 60°C for 16 h (30, 31). Hybridization conditions were previously adjusted to minimize nonspecific binding. Gels were washed with 6× SSC at room temperature for 20 min twice, followed by a 4-h room temperature wash. The gel was finally washed in 6× SSC for 3 min at 64°C, and exposed to X-OMAT AR film for 4 d.

Results

Hybridomas

Eight hybridoma cell lines from seven individuals were initially selected from 20 cell lines. All individuals demonstrated substantial increases in serum anti-Hib PS Ab 1 mo after vaccination (Table I). Hybridomas were selected to exemplify serologic characteristics (kappa or lambda expression, and K100 PS

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*Table I. Summary of Hybridoma Cell Lines**

Hybridoma	Isotype	Age	Vaccine Form	K100 Inh of mAb	Serum Anti-Hib PS Ab: Total Ab Pre	Total Ab Post	ΔK100 Inh Pre	ΔK100 Inh Post
RC3	IgA/k	Adult	PS-D	−	1.1	300	11	11
ED6.1	IgG_2/k	4 yr	PS-D	−	0.8	22	0	0
SB5/D6	IgA_1/λ	Adult	PS-D	+	2.0	688	30	27
RAY4	IgA_2/λ	Adult	PS-D	+	1.9	28	0	42
LSF2	IgA_1/λ	Adult	PS	+	ND	138	ND	29
JB32	IgA_1/λ	11 yr	PS	+	0.3	125	19	33
JB21	IgG_2/λ	11 yr	PS	−	0.3	125	19	33
16M3C8	IgG_2/λ	Adult‡	PS	−	ND	ND	ND	ND

* Columns show name of hybridoma cell line, isotype of anti-Hib PS Ab secreted, age of subject at immunization, type of vaccine (PS is plain polysaccharide, PS-D is Hib PS covalently linked to diphtheria toxoid), presence (+) or absence (−) of inhibition of binding of anti-Hib PS Ab by *E. coli* K100 PS, concentration of total serum anti-Hib PS AB pre- and 1 mo post-immunization, and percent inhibition of binding of serum lambda antibodies to Hib PS by *E. coli* K100 PS in pre- and 1 mo post-immunization sera. ‡ Pool of 3 adult donors. ND, not done.

cross-reactivity) and to represent the response to "T-independent" plain polysaccharide, or "T-dependent" polysaccharide-protein conjugate forms of antigen. (Table I).

Four lambda-expressing anti-Hib PS Ab, obtained from four different subjects, cross-reacted with K100 PS. One of these individuals, RAY4, had no detectable serum cross-reactivity before immunization. The Abs JB32 and JB21 were obtained from the same individual, and differed in heavy chain isotype and K100 cross-reactivity.

IgV_L genes. IgV_L nucleic acid sequences were obtained from two kappa and six lambda hybridomas. Candidate germline IgV_L sequences were sought by comparing hybridoma sequences to those registered with EMBL and GenBank (32).

Four lambda-expressing hybridomas, derived from four unrelated subjects, use V_λ segments which are 96–98% homologous to one another, and are members of the germline V_λVII family of which a single member, 4A, has been previously described (33) (Fig. 1). Notably, each of these four V_λVII-encoded hybridomas cross-reacts with K100 PS. The high degree of homology between these segments suggests that these genes may be encoded by a single, or group of, closely related germline elements. The four hybridoma genes share a number of nucleic acid differences (maximum nucleic acid sequence homology 89%) from the only previously sequenced V_λVII germline gene 4A, implying that these genes may be derived from another, as yet undescribed, V_λVII germline element.

The homology of the translated amino acid sequences of these 4 V_λVII sequences parallels the nucleic acid homology. The hybridoma V_λVII genes are 90 to 97% homologous at the amino acid level (Fig. 2). Framework regions are 94 to 100% homologous, whereas CDR regions are 72 to 96% homologous. Whether these minor differences reflect genetic polymorphisms, or somatic mutation, cannot be resolved from these data.

JB21 and 16M3C8, the lambda-expressing hybridomas which do not cross-react with K100 PS, are encoded by a V_λII gene family member (34), (Fig. 3). JB21 is 91% homologous to the germline V_λ2.1 gene (99% homologous in framework regions and 74% homologous in CDRs). 16M3C8 is 89% homologous to V_λ2.1 (95% in framework regions and 77% in CDRs). JB21 shares 87% amino acid homology with the translated V_λ2.1 germline sequence (99% in framework regions and 60% in CDR regions), and 16M3C8 shares 82% amino acid homology (92% in framework regions and 60% in CDRs) (Fig. 4).

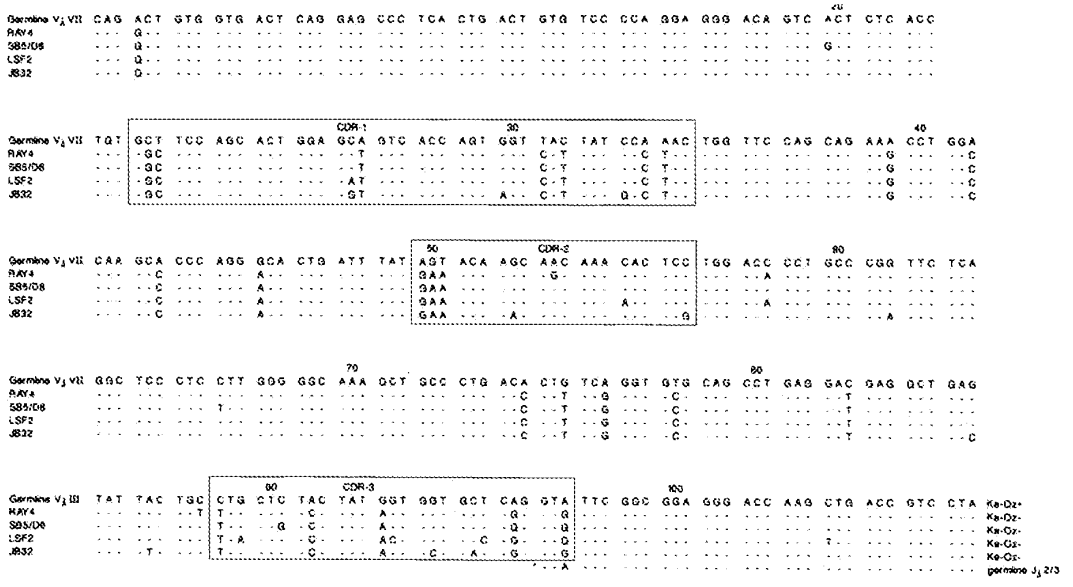

Figure 1. Nucleotide sequences of the V_λ and J_λ genes from four hybridoma cell lines (RAY4, SB5/D6, LSF2, JB32), and from the V_λVII germline gene, 4A (33). Numbering is according to Kabat (23). These sequence data are available from EMBL/GenBank Data Libraries under accession numbers M80917 (RAY4), M80918 (SB5/D6), M80915 (LSF2), and M80919 (JB32).

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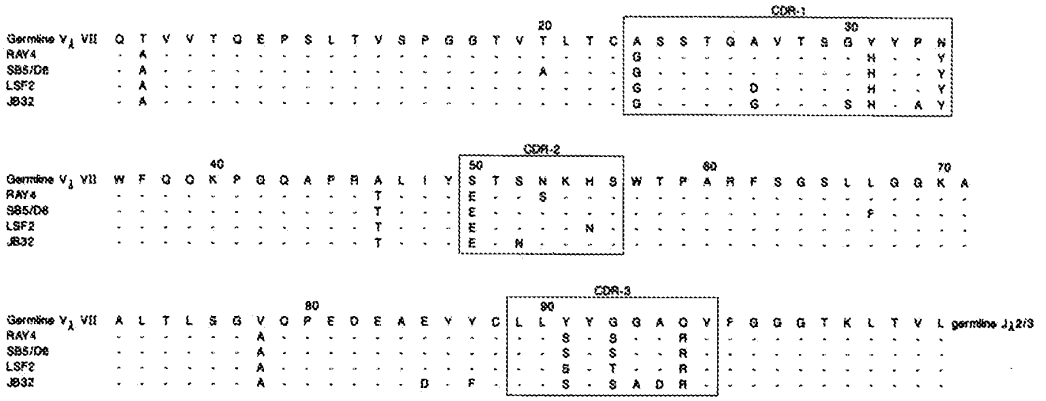

Figure 2. Translated amino acid sequences of V_λ and J_λ from four hybridoma cell lines (RAY4, SB5/D6, LSF2, JB32), and from the V_λVII germline gene, 4A (33). (See Fig. 1.)

These two V_λII-encoded gene segments are 91% homologous to one another, and have in common a number of nucleic acid differences from the V_λ2.1 germline gene (especially in CDRs). Whether they derive from another germline gene, or whether these differences reflect mutation, is unknown.

All four of the V_λVII-encoded antibodies are also encoded by V_HIII segments closely homologous to the germline gene 9.1 (5). Similar V_H segments also encode Ab utilizing other V_λ and V_κ gene segments (Table II) (manuscript in preparation). It is uncertain whether pairing of 9.1-like V_H and V_λVII-encoded heavy and light chains contributes to binding specificity or avidity. JB21 and RC3, also encoded by 9.1-like V_H gene segments, but paired with V_λII- and V_κII-encoded light chains, respectively, do not cross-react with K.100 PS.

All six of the lambda light chains are encoded by a common J_λ segment closely homologous to both J_λ2 and J_λ3 germline genes (Fig. 1) (23). J_λ2 is associated with the use of the $(Kern^-Oz^-)C_\lambda$ segment 2, and J_λ3 with the $(Kern^-Oz^+)C\lambda$ segment 3 (37). Each of these J_λ segments substitutes the codon GTG for the reported germline sequence GTA at amino acid position 97. Interestingly, this change does not result in an amino acid replacement, and is therefore not of importance in antigen binding. This minor sequence difference may reflect a shared genetic polymorphism, rather than somatic mutation.

The hybridoma RC3 uses a V_κII segment identical to the germline V_κII gene A2, and a J_κ3 segment closely homologous to the germline sequence (Fig. 5) (13). The second kappa hybridoma, ED6.1, is encoded by a member of the V_κI family

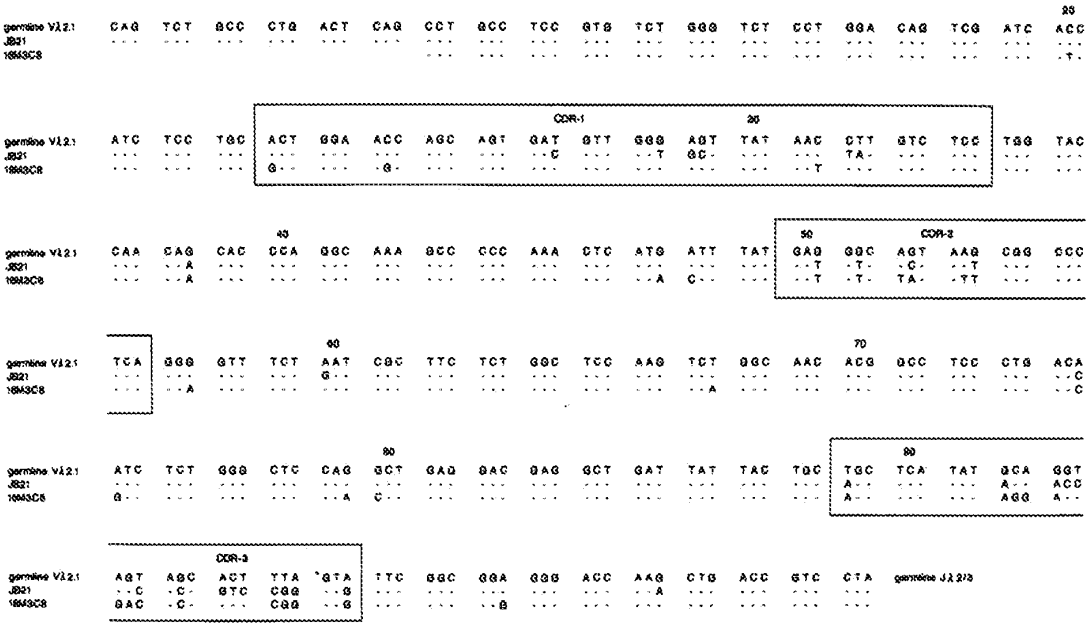

Figure 3. Nucleotide sequences of V_λ and J_λ genes from two hybridoma cell lines (JB21 and 16M3C8). Also shown is a germline gene V2.1 (34), and germline J_λ2/3 gene (23). An asterisk denotes the beginning of the J_λ segment. Numbering is according to Kabat (23). These sequence data are available from EMBL/GenBank Data Libraries under accession numbers M80916 (JB21) and M80921 (16M3C8).

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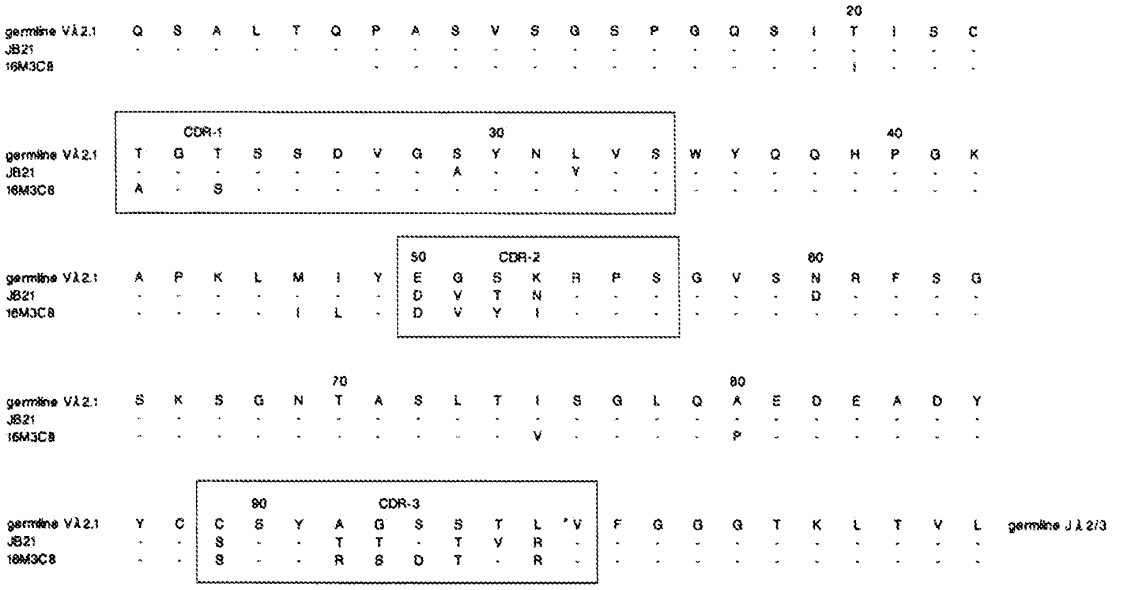

Figure 4. Translated amino acid sequences of V_λ and J_λ genes from two hybridoma cell lines (JB21 and 16M3C8). Shown for comparison is the translated sequence of the V_λ germline gene V_λ2.1, and J_λ2/3 germline gene. An asterisk denotes the beginning of the J_λ segment. (See Fig. 3.)

(36), and uses a J_κ4 segment differing from the germline sequence by a single base (Fig. 6). The ED6.1 sequence is identical to the partial amino acid sequence reported previously for 2 kappa antibodies purified from serum (12).

VJ joints of seven of these eight hybridoma V_L genes are notable for the presence of an arginine codon (Fig. 7). Comparison of germline sequences indicates that in the RC3 V_L gene, the codon CGA could only have arisen by the addition of a non–germline-encoded G at the $V_\kappa J_\kappa$ joint. In the V_λVII-encoded antibodies, the relevant germline V_λ segment is unknown. The arginine codon may be germline-encoded, or result from either mutation or N sequence addition. The VJ splice junction in all lambda antibodies is identical, implying this V_λ/J_λ combination is a necessary prerequisite for generation of the arginine. Since the CDR-3 region encodes the third hypervariable region, the frequent finding of an arginine residue at this location, and particularly its presence in both kappa and lambda anti–Hib PS Ab clonotypes, implies this residue is of considerable importance in antigen binding.

*Table II. Summary of V_H and V_L Gene Segments**

Hybridoma	V_H Segment	V_L Segment
SB5/D6	V_HIII 9.1 (5)	V_λVII 4A (33)
RAY4	V_HIII 9.1	V_λVII 4A
JB32	V_HIII 9.1	V_λVII 4A
JB21	V_HIII 9.1	V_λII 2.1 (34)
RC3	V_HIII 9.1	V_κII A2 (13)
LSF2	V_HIII 9.1	V_λVII 4A
16M3C8	V_HIII VH26 (35)	V_λII 2.1
ED6.1	N.K.	V_κI clone KC-1 (36)

* Columns show name of hybridoma cell line and most closely homologous known germline V_H and V_L segments. Reference for germline shown in parentheses. NK, Not known.

Southern blots/analysis of germline V_λVII genes

The available repertoire of V_λVII family members was examined by hybridization of human genomic DNA with the V_λVII probe generated from LSF2. 10 prominent bands, as well as an additional 5 fainter bands, were demonstrated (Fig. 8). Notably, polymorphism such as the presence or absence of a 5.0-kb fragment, and varying intensity of the 3.5-kb fragment, was demonstrated even within this relatively small sample. The human lambda variable region locus is believed to contain about 60 members, of which 10 are members of the V_λVII family (33, 38). The five less prominent bands may represent members of other V_λ families sharing significant homology with the V_λVII family. To determine if the V_λVII-encoded antibodies derive from a single germline gene, an oligonucleotide probe corresponding to conserved V_λVII CDR-1 sequence was used to identify a single 3.7-kb genomic DNA fragment. Although more than one V gene may be present in a single restriction fragment, both the repeated isolation of highly homologous V_λVII genes, and the single prominant band detected with the oligonucleotide probe, suggests that a single V_λ gene may be responsible for the generation of lambda anti–Hib PS Ab that cross-reacts with K100 PS (Fig. 9). Less prominant higher molecular weight bands are also present, but hybridize weakly to the oligomer after subtraction of nonspecific background binding by high molecular weight DNA.

Discussion

To examine the molecular basis of the immune response to Hib PS, a panel of hybridomas secreting human monoclonal anti–Hib PS Ab has been developed. Our previous studies showed exclusive use of members of the V_HIII gene family in this im-

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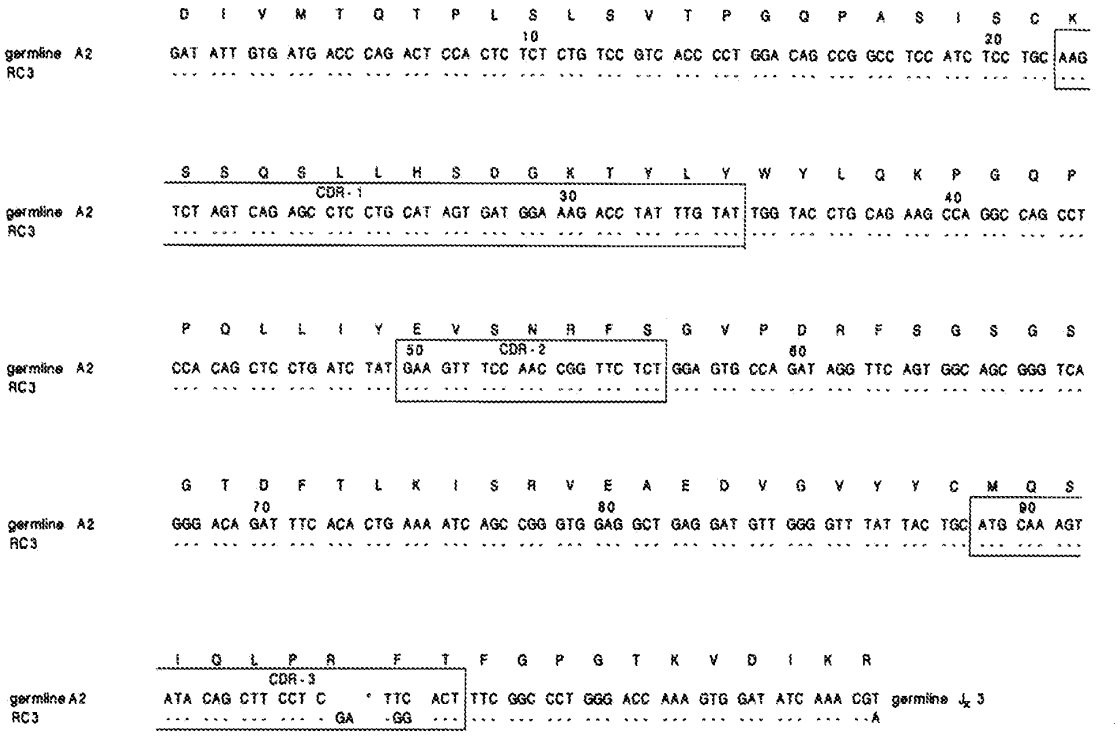

Figure 5. Nucleotide sequences of V_κ and J_κ genes from the hybridoma cell line RC3. Also shown is the translated amino acid sequence of the RC3 V_κ gene. Shown for comparison is the nucleotide sequence of a germline V_κ gene, A2 (13), and germline $J_\kappa 3$ gene. An asterisk denotes the beginning of the J_κ segment. Numbering is according to Kabat (23). This sequence data is available from EMBL/GenBank Data Libraries under accession number M80914 (RC3).

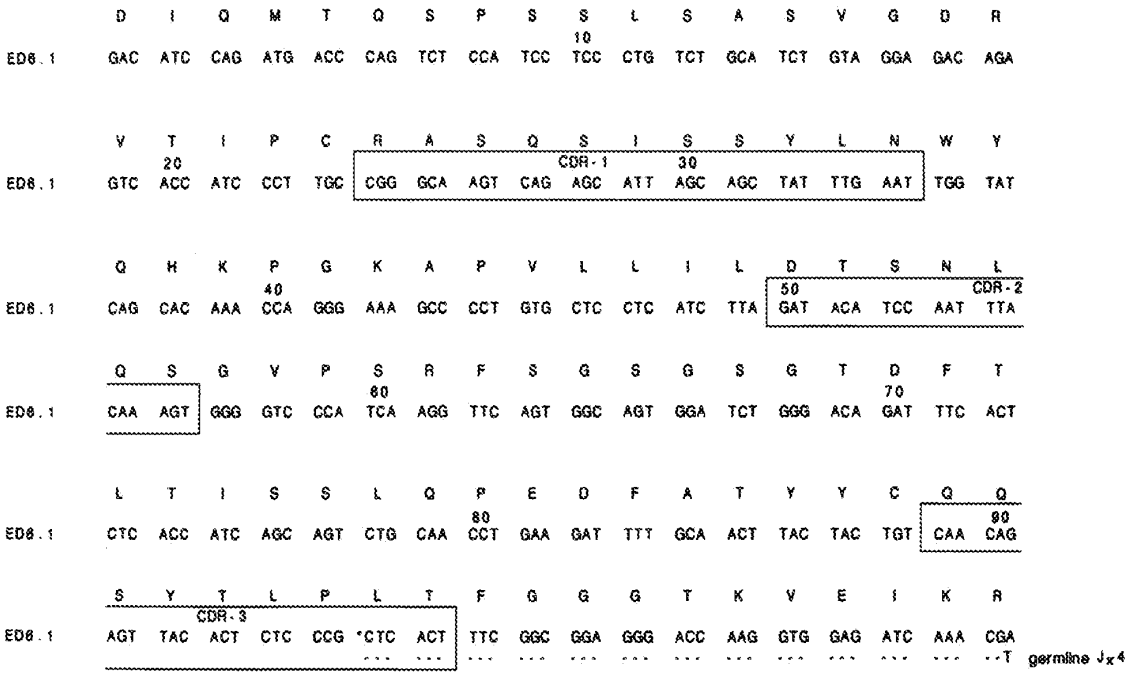

Figure 6. Nucleotide sequence of V_κ and J_κ genes from hybridoma cell line ED6.1. Also shown is the translated amino acid sequence of the ED6.1 V_κ gene. An asterisk denotes the beginning of the J_κ segment. Numbering is according to Kabat (23). This sequence data is available from EMBL/GenBank Data Libraries under accession number M80920 (ED6.1).

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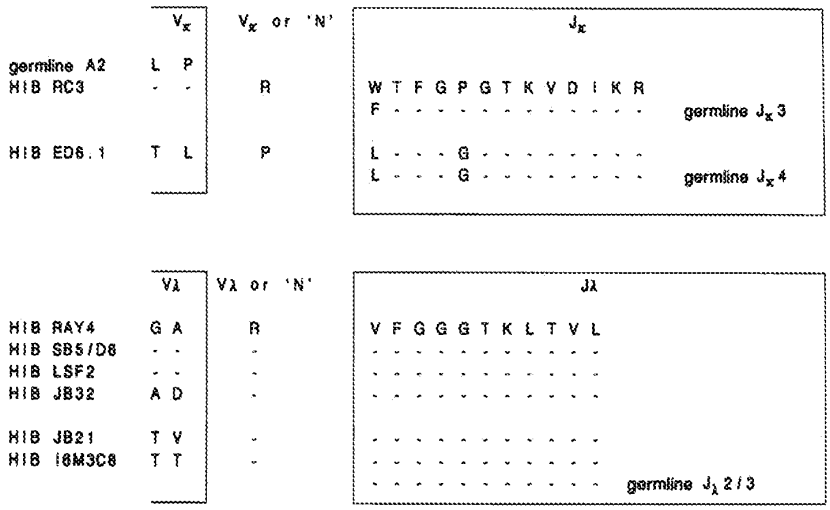

Figure 7. Translated amino acid sequences of VJ joints from seven hybridoma cell lines (RC3, ED6.1, RAY4, SB5/D6, LSF2, JB21, and 16M3C8). Also shown are translated amino acid sequences of the germline V_κ gene A2, and germline $J_\kappa 3$, $J_\kappa 4$, and $J_\lambda 2/3$ genes. (See Figs. 2, 4, and 6.)

mune response, and the apparent restriction to two or three germline V_H genes. In contrast, V_L gene usage is more complex, with antibody encoded by a number of V_λ and V_κ families (12). Analysis of light chain variable region genes obtained from cell lines selected to represent various characteristics of serum antibodies showed that despite diversity in the variety of light chain variable region gene segments utilized, most serum anti–Hib PS Abs may be encoded by a limited number of V_κ and V_λ germline genes.

The light chain distribution of anti–Hib PS Ab is characterized by a predominance of kappa light chains, although some individuals predominantly or exclusively express lambda light chains (39, 40). Scott et al. have shown that V_κII light chains are the most commonly expressed light chains among clonally purified serum anti–Hib PS antibodies from adults with high responses to immunization. Limited amino acid sequences of a number of these serum antibodies showed that greater than 60% of subjects immunized with plain Hib PS produce antibody encoded by a V_κII family member, and further, that a number of these antibodies show identical amino acid homology with the translated amino acid sequence of the germline V_κII gene A2 (12, 13). Lucas et al. have described an anti-idiotypic monoclonal antibody (Hibid-1) that identifies anti–Hib PS antibodies expressing this light chain (16). On average, 60%

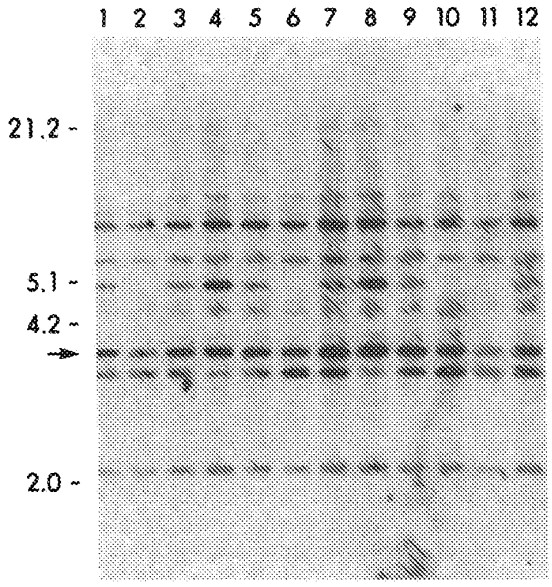

Figure 8. Southern blot analysis for V_λVII family members in BamHI-digested human genomic DNA. Lane *12* contains genomic DNA from the subject from whom hybridoma LSF2 was obtained. Lane *9* contains genomic DNA from a parent of LS. The remaining 10 donors are unrelated. An arrow indicates the 3.7-kb fragment which is identified by the oligonucleotide probe illustrated in Fig. 9.

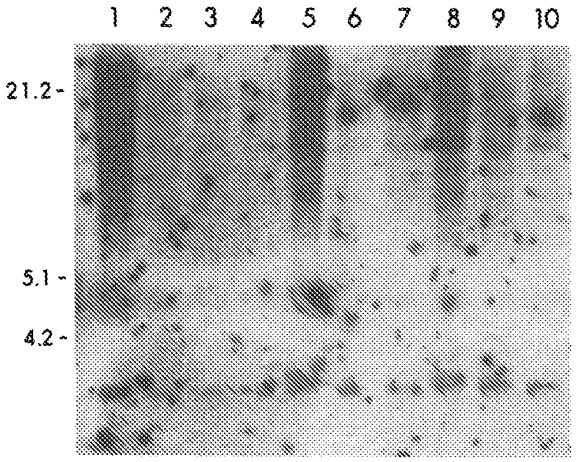

Figure 9. Dried gel hybridization of BamHI-digested human genomic DNA with an oligonucleotide probe corresponding to conserved V_λVII hybridoma CDR-1 sequences. Genomic DNA in lanes *1, 4, 5, 6, 7, 9* and *10* of Fig. 8 corresponds to that in lanes *2, 7, 4, 8, 10, 5* and *9* of Fig. 9, respectively.

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of the total anti–Hib PS antibody of most children and adults immunized with either Hib PS or Hib PS-conjugate vacine expresses this idiotype (15, 16). Taken together, this suggests the most common clonotype of anti–Hib PS Ab is encoded by a single V_κII gene. The V_κ locus contains approximately 50 functional genes, of which 40% are members of the V_κII family (41). The V_κII A2 germline gene is known to be present in a single copy (13). Our findings definitively demonstrate that at least a portion of V_κII-encoded antibodies are encoded by unmutated germline elements. Our subject R.C. was immunized with PS-protein conjugate vaccine, whereas Scott's subjects were immunized with the plain PS vaccine. Thus it is possible that the unmutated V_κII A2 segment is utilized irrespective of antigen presentation in a T-independent or T-dependent form.

Lambda variable region gene usage appears similarly restricted. The majority of lambda-expressing anti–Hib PS Ab in adults with high antibody responses to vaccine cross-reacts with *E. coli* K100 PS (17). The capsular polysaccharides of Hib and *E. coli* K100 are structurally similar and gastrointestinal colonization with *E. coli* K100 induces antibody to Hib PS in humans (42). "Naturally occurring" anti–Hib PS Ab in children commonly cross-reacts with K100 PS, whereas Ab of individuals immunized with Hib PS or responding to Hib infection is less frequently cross-reactive (18). The kappa/lambda ratio of natural anti–Hib PS Ab is lower in infants than in older children, and in the vaccine-induced antibody of adults (39). Further, Hibid-1 (the CRI associated with expression of the V_κII light chain) is expressed less frequently and comprises a smaller proportion of total anti–Hib PS Ab in preimmune compared with postimmunization sera. Expression of Hibid-1 following immunization is similar in adults and children. These results imply that cross-reactive antigens may induce the natural antibody of children. K100 PS-cross-reactive antibody may provide protective immunity in young children upon first exposure to Hib. These cross-reactive antibodies appear to be subsequently replaced or dominated by antibody specific for Hib PS when individuals are exposed to this antigen by immunization, colonization, or infection. We demonstrate that cross reactivity of lambda antibodies may be mediated through a single light chain germline gene. The mechanism for the clonal dominance of the V_κII antibodies is unclear; the avidity for Hib PS of these K100 PS–cross-reactive antibodies from adults is similar to that of Hib specific PS antibodies (P. Shackelford, unpublished observation).

Lambda antibodies expressing the V_λII gene did not cross-react with *E. coli* K100 PS. Whether all noncross-reactive antibodies are encoded by closely related members of the V_λII gene family will require further characterization of additional antibodies.

By the age of about 5 yr most individuals have acquired protective anti–Hib PS Ab. All of the hybridomas in this study were derived from older children or adults, who had detectable preimmunization antibody. The B cells immortalized by fusion, therefore, most likely represent a secondary immune response. Despite this, V_H gene segments obtained from these hybridomas appear to be relatively unmutated (14), and it appears that some V_L genes are similarly conserved. The RC3 V_κ gene segment is identical to the A2 germline gene, and although the pertinent V_λVII germline gene is not known, the paucity of differences in V_λVII segments from four different subjects suggests these gene segments are relatively unmutated. J_κ and J_λ segments are also highly homologous to germline elements. Thus, at least a portion of the anti–Hib PS response represents the use of unmutated germline elements.

VJ joints of the majority of rearranged V_L genes also appear to be an additional source of restriction in the anti–Hib PS immune response. Although there is variability in the use of J_κ segments, V_κII-encoded V_L genes are characterized by the presence of an arginine at amino acid position 95a (13). In the RC3 V_L gene and at least one of the antibodies (B-G2a) described by Scott, this appears to have arisen by N sequence addition. In four other kappa antibodies described by Scott, alternative joining may have generated this invariant arginine (13). All of the lambda V_L genes in the present study are similarly characterized by an invariant arginine-96. The germline V_λVII segment is not known; therefore the mechanism used to generate this codon is uncertain. The invariant presence of this residue in many anti–Hib PS Abs supports the previous suggestion that it may be of great importance in antigen binding (13). This also is supported by studies of the murine anti-*p*-azophenylarsonate (Ars) response. Anti-Ars light chains also contain an invariant arginine at position 96, encoded by intracodonic splicing of a single V_κ (V_κ10-Ars) and a single J_κ (J_κ1) segment (43). This arginine is essential for Ars binding, and the substitution of tyrosine for arginine has been shown to abolish binding (44). The presence of arginine residues clustered within the CDR's of the heavy chains of murine anti–DNA antibodies correlates with increased affinity, and the arginine requirement in the anti–Hib PS response may indicate its participation in the recognition of the similar phosphate–ester linkage of the repeating carbohydrate moiety (45). Thus, although diversity exists in the use of a variety of V and J segments, certain VJ combinations, which produce arginine codons by junctional flexibility or N segment addition, may be strongly selected.

Does the restricted nature of V_H and V_L segment usage in the human anti–Hib PS response explain the age-related acquisition of immunity to this organism? In both mice and humans, preferential use of certain V_H gene segments is observed in early development. In mice, biased usage of J_H-proximal V_H gene segments occurs in fetal and neonatal animals (46, 47). Developmental regulation of V_L gene usage is less extensively studied than V_H genes. Teale and Morris noted preferential expression of certain V_κ families in LPS-stimulated murine fetal B cells compared to adult B cells, with no evidence for position-dependent bias (48). Kaushik et al. also described nonstochastic use of V_κ families in adult mice, suggesting molecular constraints may persist beyond the neonatal period (49). Two or three V_H gene segments, and a single V_κ and a single V_λ gene segments, appear to encode a large proportion of anti–Hib PS Ab in immune subjects. The use of highly conserved V gene segments in human anti–Hib PS response may place considerable restraints on the immune response. The inability to utilize these genes, whether due to chromosomal position or other molecular constraints, may be responsible for the age-related susceptibility to Hib infection. The lack of somatic hypermutation in a portion of these antibodies would also place greater dependence on the germline repertoire, in that it may be impossible to improve antibody affinity by antigen-driven selection of favorable mutations. The absence or polymorphism of these genes also may contribute to individual and ethnic susceptibility to invasive Hib disease. In addition, the repertoire differences detected in some individuals (such as the absence of kappa antibody, or the absence of the V_κII-associated CRI) may be explained by the use of other minor light chains. An individ-

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ual missing the A2 germline gene has been described, although the functional implications of such a deletion are not known (13).

Expression of certain V_H gene family members in mice is correlated with B cell subpopulations (50). An alternative explanation for the poor response to plain Hib PS may be a maturational delay in recruitment of a critical B cell population that selectively expresses important variable region gene segments. We and others note that an arginine is expressed at the VJ joint in the great majority of anti-Hib PS light chains. In some circumstances N segment addition is required to generate this codon. The addition of these extra nucleotides is infrequent in light chain recombination, suggesting that these B cell precursors may be relatively infrequent (51). Moreover, N segment addition also appears to be developmentally regulated. Neonatal murine B cells lack N sequences, and their presence in heavy chain recombination increases with time (52). In addition, N sequence addition may also correlate with certain B cell subsets. N segments appear to be rare in Ly1-B cell-derived populations (53).

In contrast to plain polysaccharide vaccines, most young infants respond well to the newer Hib-PS protein conjugate vaccines (54). It is presently unknown if this antigen formulation recruits an alternative antibody repertoire, or if, by an uncertain mechanism, developmental restriction of a "dormant" B cell subset is overcome. To address this question, V gene expression in infants must be examined.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888

Confirmation No.: 2558

Filed: September 9, 2016

Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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Dated: November 4, 2016

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

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Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

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- 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 *10/3/2016*.

☐ 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) *1* 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) *1* 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/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *10/21/2016*.

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

4) ☐ Other: _____.

DETAILED ACTION

1. Claim 1 is pending and under consideration in the instant examination.

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

3. Applicant's IDS's, filed 10/21/2016, are acknowledged and have been considered.

Double Patenting

4. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., In re Berg, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); In re Goodman, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); In re Van Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); In re Thorington, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

5. Claim 1 is provisionally rejected on the ground of nonstatutory obviousness- type double patenting as being unpatentable over claim 1 of copending **Application No. 15/148,839**.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because the specification of the '839 at ¶158 defines eculizumab as having a heavy chain SEQ ID NO: 2 and light chain SEQ ID NO: 4 which is 100% identical in amino acid sequence to the currently claimed SEQ IDs NO: 2 and 4. (see alignment below).

SEQ ID NO: 2 alignment

```
Qy          1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY 60

Qy         61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV 120

Qy        121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ 180

Qy        181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS 240

Qy        241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST 300

Qy        301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT 360

Qy        361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE 420

Qy        421 GNVFSCSVMHEALHNHYTQKSLSLSLGK 448
              ||||||||||||||||||||||||||||
Db        421 GNVFSCSVMHEALHNHYTQKSLSLSLGK 448
```

SEQ ID NO: 4 alignment

```
Qy          1 MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQ 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQ 60
```

```
Qy          61 KPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNVLNTPLTF 120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          61 KPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNVLNTPLTF 120

Qy         121 GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN 180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         121 GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN 180

Qy         181 SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 236
               |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         181 SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 236
```

6. The closest prior art was regarded to be Hillmen et al. (N Engl J Med 2004; 350: 552-9). Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶). However, Hillmen does not teach the specific anti-C5 antibody recited by the current application. As evidenced by Report from Alexion Pharma K.K. eculizumab has a H and L chain sequence (p. 3 of the Report) which when compared to the H and L chains of the currently recited anti-C5 antibody are not the same (see sequence alignment below ExPASy (userSeq1 is the imputed sequence of eculizumab and userSeq2 is the amino acid sequence for the H chain of the currently recited claims).

```
98.9% identity in 448 residues overlap; Score: 2364.0; Gap frequency: 0.0%

UserSeq1       1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY
UserSeq2       1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY
                 ************************************************************

UserSeq1      61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV
UserSeq2      61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV
                 ************************************************************

UserSeq1     121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
UserSeq2     121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
                 ************************************************************

UserSeq1     181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS
UserSeq2     181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS
                 ************************************************************

UserSeq1     241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
UserSeq2     241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHANKTKPREEQFNST
                 *********************************************  ************

UserSeq1     301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
UserSeq2     301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPEPEQVYTLPPSQEEMT
                 *********************************************   *************

UserSeq1     361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE
UserSeq2     361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE
                 ************************************************************

UserSeq1     421 GNVFSCSVMHEALHNHYTQKSLSLSLGK
```

```
UserSeq2      421 GNVFSCSVMHEALHNHYTQKSLSLSLGK
                  ****************************
```

One of skill in the art would not have been easily guided to using the currently claimed anti-C5 antibody for treating a patient suffering from PNH given the teachings of Hillmen.

7. No claim is allowed.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

October 26, 2016

/JAMES ROGERS/
Patent Examiner, AU 1644

/SHARON WEN/
Primary Examiner, Art Unit 1644

Electronic Patent Application Fee Transmittal

Application Number:	15260888
Filing Date:	09-Sep-2016
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

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

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
STATUTORY OR TERMINAL DISCLAIMER	1814	1	160	160
		Total in USD ($)		**160**

Electronic Acknowledgement Receipt	
EFS ID:	27408277
Application Number:	15260888
International Application Number:	
Confirmation Number:	2558
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Milejdi Sartori
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3
Receipt Date:	04-NOV-2016
Filing Date:	09-SEP-2016
Time Stamp:	12:29:57
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$160
RAM confirmation Number	110416INTEFSW00009265120080
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Assignee showing of ownership per 37 CFR 3.73	AXJ_114BUSCN3_373.pdf	2049281 a4c7c337718c6a3c6e0a3a26d3435dc34287644a	no	2
Warnings:					
Information:					
2	Terminal Disclaimer Filed	AXJ_114BUSCN3_TD.pdf	1745266 6759a2a3b09aa1d05d767bd83823d328ac516dd0	no	2
Warnings:					
Information:					
3	CRF Statement Paper and CRF are the same	AXJ_114BUSCN3_Statement_re_SL.pdf	110454 b451f77311ef23d93894571a681f9b45f1d7a6bc	no	2
Warnings:					
Information:					
4	Sequence Listing (Text File)	AXJ_114BUSCN3_Seq_Listing.txt	10282	no	-
Warnings:					
Information:					
5	Amendment/Req. Reconsideration-After Non-Final Reject	AXJ-114BUSCN3_Response_to_Office_Action.pdf	152222 0e2137d2cc3f4ea9102e08ffd687fe3e9ba661ec	no	7
Warnings:					
Information:					
6	Non Patent Literature	AXJ_114BUSCN3_Exhibit_A.pdf	17187219 84a800065b3a719f77df23c93c8f2bb452526c1b	no	11
Warnings:					
Information:					

7	Information Disclosure Statement (IDS) Form (SB08)	IDSTRANS.pdf	27548 fb353dc1286a2bf752c7b419ee6f93eba497979c	no	3
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
8	Other Reference-Patent/App/Search documents	15284015.pdf	297399 5ce401f4c66470c65ab8ad5f407ec6f4d0b9c717	no	6
Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	30405 7e80102450db723ce5e70ca3663e3432d8aaeade	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	21610076		

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

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

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

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

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

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Alexion Pharmaceuticals, Inc.

Application No./Patent No.: 15/260,888 Filed/Issue Date: September 9, 2016

Titled: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Alexion Pharmaceuticals, Inc. (Name of Assignee) , a Corporation (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

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

1. [X] The assignee of the entire right, title, and interest.

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

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

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

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

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

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

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

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

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

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

1. From: Leonard Bell To: Alexion Pharmaceuticals, Inc.
   The document was recorded in the United States Patent and Trademark Office at Reel 039718, Frame 0748, or for which a copy thereof is attached.

2. From: Russell P. Rother To: Alexion Pharmaceuticals, Inc.
   The document was recorded in the United States Patent and Trademark Office at Reel 039718, Frame 0748, or for which a copy thereof is attached.

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

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

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

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

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

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

Signature	Date
11/2/16	
Todd Spalding	Vice President, Head of Intellectual Property
Printed or Typed Name	Title or Registration Number

PTO/AIA/25 (04-13)
Approved for use through 04/30/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION	Docket Number (Optional) AXJ-114BUSCN3

In re Application of: Leonard BELL *et al.*

Application No.: 15/260,888-Conf. #2558

Filed: September 9, 2016

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

The applicant, Alexion Pharmaceuticals, Inc., owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending **reference** Application Number (see attached), filed, _____, as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the **reference** application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said **reference** application, "as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application," in the event that: any such patent granted on the pending **reference** application expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

Check either box 1 or 2 below, if appropriate.

1. [X] The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

2. [ ] The undersigned is an attorney or agent of record. Reg. No. _____

Signature

11/2/2016
Date

Todd Spalding
Typed or printed name

Vice President, Head of Intellectual Property
Title

Telephone Number

[X] Terminal disclaimer fee under 37 CFR 1.20(d) is included.

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.

SUPPLEMENTAL SHEET FOR TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION	Docket Number (Optional) AXJ-114BUSCN3

Reference application Nos. applicable to this terminal disclaimer (referenced on first page):

15/148,839 (filed May 6, 2016)

15/284,015 (filed October 3, 2016)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: November 4, 2016
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888 Confirmation No.: 2558

Filed: September 9, 2016 Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

MS Sequence
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT PURSUANT TO 37 CFR 1.821

Dear Sir:

Submitted herewith in connection with the above-referenced patent application and in full compliance with 37 C.F.R. §§1.821-1.825 is a computer readable copy and paper copy of the Sequence Listing (filed together as a .txt file via the United States Patent Office's Electronic Filing System).

I hereby state that I have reviewed the paper copy of the Sequence Listing, as required by 37 CFR 1.821(c), and have reviewed the computer readable form of the Sequence Listing, as required by 37 CFR 1.821(e), and that the content of the paper and computer readable copies for the above-referenced Patent application are the same as required by 37 CFR 1.821(f) (note that these documents are submitted as one electronic file). No new matter has been added to the Sequence Listing.

Dated: November 4, 2016

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 15260888 Document Date: 11/04/2016

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

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

- Sequence Listing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via eDAN using the Supplemental Content tab, or via the SCORE web page.
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Form Revision Date: August 26, 2013

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3	2558

133485 7590 11/14/2016

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
11/14/2016	ELECTRONIC

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

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

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

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	15/260,888	BELL ET AL.	
	Examiner	Art Unit	
	JAMES ROGERS	1644	

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

(1) *JAMES ROGERS*.

(2) *DANIEL KOLKER, SPE*.

(3) *JANE REMILLARD*.

(4) *DARRELL FONTENOT*.

Date of Interview: *01 November 2016*.

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: *1*.

Identification of prior art discussed: _____.

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...)

Proposed possible amendments to claim 1 were discussed. No agreement was reached but applicant agreed to submit an amendment/response to the prior office action.

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

/JAMES ROGERS/ Examiner, Art Unit 1644	/DANIEL E. KOLKER/ Supervisory Patent Examiner, Art Unit 1644

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.

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============================================================
Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).
Reviewer: Douglas, Keisha (ASRC)
Timestamp: [year=2016; month=11; day=10; hr=16; min=49; sec=12; ms=286;
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Validated By CRFValidator v 1.0.5

Application No: 15260888 Version No: 2.0

Input Set:

Output Set:

Started: 2016-11-04 12:31:19.211
Finished: 2016-11-04 12:31:19.978
Elapsed: 0 hr(s) 0 min(s) 0 sec(s) 767 ms
Total Warnings: 10
Total Errors: 0
No. of SeqIDs Defined: 10
Actual SeqID Count: 10

Error code		Error Description
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SEQUENCE LISTING

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<120> TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS
BY AN INHIBITOR OF COMPLEMENT

<130> AXJ-114BUSCN3

<140> US 15/260,888
<141> 2016-09-09

<150> 15/148,839
<151> 2016-05-06

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<151> 2012-03-22

<150> 12/225,040
<151> 2009-05-13

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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ile Phe Ser Asn Tyr
20 25 30

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

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Glu Tyr Thr Glu Asn Phe
50 55 60

Lys Asp Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

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

Ala Arg Tyr Phe Phe Gly Ser Ser Pro Asn Trp Tyr Phe Asp Val Trp
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Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
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            20                  25                  30

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

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Glu Tyr Thr Glu Asn Phe
    50                  55                  60

Lys Asp Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65                  70                  75                  80

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

Ala Arg Tyr Phe Phe Gly Ser Ser Pro Asn Trp Tyr Phe Asp Val Trp
            100                 105                 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
        115                 120                 125

Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr
    130                 135                 140
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Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145                 150                 155                 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
                165                 170                 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
            180                 185                 190

Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp
        195                 200                 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys
    210                 215                 220

Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser
225                 230                 235                 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro
            260                 265                 270

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val
    290                 295                 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr
                325                 330                 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            340                 345                 350

Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
        355                 360                 365
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Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370                 375                 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390                 395                 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser
                405                 410                 415

Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
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Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gly Ala Ser
        35                  40                  45

Glu Asn Ile Tyr Gly Ala Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
    50                  55                  60

Ala Pro Lys Leu Leu Ile Tyr Gly Ala Thr Asn Leu Ala Asp Gly Val
65                  70                  75                  80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
                85                  90                  95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Asn
            100                 105                 110
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20 25 30

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

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

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Asn Val Leu Asn Thr Pro Leu
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln

145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

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Phe Asn Arg Gly Glu Cys
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1 5

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peptide

<400> 10
Gln Asn Val Leu Asn Thr Pro Leu Thr
1 5

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

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/260,888	Filing Date 09/09/2016	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

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

APPLICATION AS AMENDED – PART II

		(Column 1)		(Column 2)	(Column 3)		
AMENDMENT	11/04/2016	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 10	Minus	** 20	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**0**

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

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** 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.

LIE
GYZELL P. JOHNSON SMITH

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Nov 14, 2016 03:31:27 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:
The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
15260888	INTV.SUM.APP	11/14/2016	AXJ-114BUSCN3

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
	15/260,888	BELL ET AL.	

Document Code - DISQ	Internal Document – DO NOT MAIL

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed : 11/4/16	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:
JEAN PROCTOR

U.S. Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
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15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3	2558

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Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
12/22/2016	ELECTRONIC

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

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

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

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 15/260,888	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	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* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

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

Status

1)☒ Responsive to communication(s) filed on *11/4/2016*.

☐ 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) *1-10* 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) *1-10* 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/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *10/21/2016, 11/4/2016*.

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

4) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-10 pending and under consideration in the instant examination.

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

3. Applicant's IDSs filed on 10/21/2016 and 11/4/2016 are acknowledged and have been considered.

4. Applicant has overcome the ODP rejections which were in the prior OA mailed on 11/4/2016 given applicant's approved TD over US Patent Application 15/148,839 (Note: applicant's has also filed an approved TD over US Patent application 15/284,015).

Claim Rejections - 35 USC § 102

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

> A person shall be entitled to a patent unless –
>
> (b) *the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.*

6. Claims 1-2 and 7-10 are rejected under 35 U.S.C. 102(b) as being anticipated by Hillmen et al. (N Engl J Med 2004; 350: 552-9, IDS Ref. of 10/21/2016), as is evidenced by applicant's specification as filed at p.44 and Thomas et al. (Molecular Immunology, 33(17/18), pp.1389-1401, 1996).

With respect to base claim 1, Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶).

While Hillmen does not specifically recite that the antibody that binds C5 comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO:4, the specification as filed at p. 44 discloses that SEQ ID NO: 2 and SEQ ID NO: 4 constitute the H and L chains for "eculizumab" and Hillmen, as noted supra, teaches administering "eculizumab". Hillmen further teaches that "eculizumab" is a recombinant humanized antibody that binds to C5 (p. 553, 3rd ¶) and cites Thomas which in turn describes in detail humanization of the clone referred to as "5G1.1" arrive at the humanized h5G1.1 which retain both the affinity and blocking functions of the murine 5g1.1 antibody (abstract). Accordingly and in absence of evidence to the contrary, the

currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the L and H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Hillmen.

Claim 2 is included because Hillmen teaches that the patients received eculizumab by infusion (p. 554 under "Treatment Schedule").

Claim 7 is included because Hillmen teaches that the patients had previously been diagnosed of aplastic anemia (p. 555, 1st ¶).

Claim 8 is included because Hillmen teaches that patients received infusions of eculizumab (600 mg) every week for four weeks, followed one week later by a 900 mg dose and then by a dose of 900 mg every other week through week 12 (p. 552 under "methods" and p. 554 under "treatment schedule").

Claims 9-10 are included because Hillmen teaches that a decrease in lactate dehydrogenase began after a single dose of eculizumab (immediate decrease occurs within 1 week of administration of the antibody) in all patients and remained within or just above the normal range for the duration of the study (sustained decrease in mean levels of LDH) (p. 555, last ¶).

The reference teachings anticipate the claimed invention.

Claim Rejections - 35 USC § 103

7. 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 even 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

8. Claims 1-4 and 7-10 rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 552-9), in view of Thomas et al. (Molecular Immunology, 33(17/18), pp.1389-1401, 1996) and of Evans et al. (US 6,355,245 B1, issued 3/12/2002).

The prior art teachings of Hillmen are discussed above.

To the extent that applicant intends to argue that Hillmen on its own does not constitute an enabling references because it does not explicitly place the eculizumab antibody in the public domain by not enabling one of ordinary skill in the art to make and use eculizumab comprising SEQ ID NO: 2 and 4 as currently claimed, Hillmen discloses (p. 553, 3rd¶) that eculizumab is a recombinant human antibody that binds to C5, and refers to reference number 15 (Thomas) as disclosing more information about eculizumab. Thomas in turn discloses that eculizumab is the humanized antibody h5G1.1. The '245 patent further discloses and claims the antibody made by hybridoma 5G1.1 (see for example claims 20-21) and thus places the 5G1.1 hybridoma and the antibody produced by it into the public domain.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to follow the teachings of Hillmen, Thomas and the ‘245 patent to arrive at the currently claimed invention. Hillmen and Thomas collectively teach that the 5G1.1 antibody can be used to treat PNH, and the ‘245 patent provides the public with this antibody. Thus one of ordinary skill in the art would have found it obvious to use this publicly available antibody in the methods disclosed by Hillmen.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

9. Claims 1-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 552-9), in view of Thomas et al. (Molecular Immunology, 33(17/18), pp.1389-1401, 1996) and of Evans et al. (US 6,355,245 B1, issued 3/12/2002), as applied to claims 1-4 and 7-10 above, and further in view of Wang (US 2005/0271660).

The prior art teachings of Hillmen, Thomas and the ‘245 patent are discussed supra.

The prior art teachings differ from the claimed invention in the recitation that the antibody is administered at a dosage level of between 5-50 mg/kg per patient per

treatment as in claim 3, that the composition is in a single unit dosage form as in claim 4 and that the single unit dosage form is a 300 mg single unit dosage form as in claim 5.

The prior art teachings further differ in the recitation that the pharmaceutical composition comprises a 300 mg single-use formulation of 30 ml of a 10 mg/ml sterile, preservative free solution as in claim 6.

The ‘245 patent further teaches antibody 5G1.1, which is the same as eculizumab (discussion supra), is effective at doses of 2.2, 3, or 5 mg per kg body weight; this corresponds to 154, 210, or 350 mg of antibody for a 70kg adult human (‘245 patent, column 16 lines 10-30). Evans (the ‘245 patent) also teaches that eculizumab is therapeutically effective when as little as 210 mg are present (column 16 lines 10-15). Evans teaches that the antibodies are particularly advantageous in that they are highly active even when administered in low doses (col 8 lines 25-32), providing a reasonable expectation of success in treating PHN with a dose less than what was disclosed by Hillmen. The reference teaches that it is within the skill of an artisan, particularly a physician, to determine the optimal dose (column 18 lines 15-30). Evans teaches formulations of the anti-C5 antibodies may be formulated in a pharmaceutically effective carrier such saline, which is considered to be a "preservative free solution". (col 18 lines 30-45).

Neither Hillmen nor the ‘245 patent (Evans) teaches a 300 mg single unit dosage as recited in the claims.

Nevertheless it would have been obvious to one of ordinary skill in the art to modify the teachings of Hillmen to administer 300 mg single unit dosages. Hillmen teaches that both 600 and 900 mg dosages can be used, and since Evans patent teaches that 210 or 350 mg of antibody would be expected to be therapeutically effective, arriving at the 300 mg dosage would be routine, convenient and obvious. One of ordinary skill in the art would have a reasonable expectation of success, as Evans teaches that the antibody exerts its effect at low doses.

It is further noted, [I]t is not inventive to discover the optimum or workable ranges by routine experimentation.” In re Aller, 220 F.2d 454, 456 (CCPA 1955); see also In re Peterson, 315 F.3d 1325 (Fed. Cir. 2003). “Only if the ‘results of optimizing a variable’ are ‘unexpectedly good’ can a patent be obtained for the claimed critical range.” In re Geisler, 116 F.3d 1465, 1469 (Fed. Cir. 1997) (quoting In re Antonie, 559 F.2d 618, 620 (CCPA 1977)). “[Discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art.” In re Boesch, 617 F.2d 272, 276 (CCPA 1980).

Finally, **Wang** teaches that eculizumab is highly soluble, including at up to 30 mg per ml (paragraph 0171, Figure 10). Wang further teaches that pharmaceutically acceptable carriers include pyrogen-free water may be used in formulation of the antibody (¶71)

(sterile, preservative free solution). Therefore the artisan of ordinary skill would have had a reasonable expectation of success in making a 10 mg / ml solution of the antibody sterile, preservative free solution.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

10. No claim is allowed.

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

December 5, 2016 /JAMES ROGERS/
Art Unit 1644

/DANIEL E. KOLKER/

Supervisory Patent Examiner, Art Unit 1644

Notice of References Cited	Application/Control No. 15/260,888	Applicant(s)/Patent Under Reexamination BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-6,355,245 B1	03-2002	Evans; Mark J.	C07K14/472	424/130.1
*	B	US-2005/0271660 A1	12-2005	Wang, Yi	C07K16/18	424/144.1
	C	US-				
	D	US-				
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	G	US-				
	H	US-				
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	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

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

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Thomas et al. "Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv" Molecular Immunology, 33(17/18), pp. 1389-1401, 1996
	V	
	W	
	X	

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

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PII: S0161-5890(96)00078-8

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INHIBITION OF COMPLEMENT ACTIVITY BY HUMANIZED ANTI-C5 ANTIBODY AND SINGLE-CHAIN Fv.

THOMAS C. THOMAS,* SCOTT A. ROLLINS, RUSSELL P. ROTHER, MICHELLE A. GIANNONI, SANDRA L. HARTMAN, EILEEN A. ELLIOTT, STEVEN H. NYE, LOUIS A. MATIS, STEPHEN P. SQUINTO AND MARK J. EVANS†

Alexion Pharmaceuticals, 25 Science Park, New Haven, CT 06511, U.S.A.

(*Received* 1 *July* 1996; *accepted in revised form* 6 *September* 1996)

Abstract—Activation of the complement system contributes significantly to the pathogenesis of numerous acute and chronic diseases. Recently, a monoclonal antibody (5G1.1) that recognizes the human complement protein C5, has been shown to effectively block C5 cleavage, thereby preventing the generation of the pro-inflammatory complement components C5a and C5b-9. Humanized 5G1.1 antibody, Fab and scFv molecules have been produced by grafting the complementarity determining regions of 5G1.1 on to human framework regions. Competitive ELISA analysis indicated that no framework changes were required in the humanized variable regions for retention of high affinity binding to C5, even at framework positions predicted by computer modeling to influence CDR canonical structure. The humanized Fab and scFv molecules blocked complement-mediated lysis of chicken erythrocytes and porcine aortic endothelial cells in a dose-dependent fashion, with complete complement inhibition occurring at a three-fold molar excess, relative to the human C5 concentration. In contrast to a previously characterized anti-C5 scFv molecule, the humanized h5G1.1 scFv also effectively blocked C5a generation. Finally, an intact humanized h5G1.1 antibody blocked human complement lytic activity at concentrations identical to the original murine monoclonal antibody. These results demonstrate that humanized h5G1.1 and its recombinant derivatives retain both the affinity and blocking functions of the murine 5G1.1 antibody, and suggest that these molecules may serve as potent inhibitors of complement-mediated pathology in human inflammatory diseases.
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Key words: complement, C5, humanized antibody, Fab, scFv.

INTRODUCTION

Activated components of the complement system are believed to contribute to many of the pathophysiological mechanisms underlying numerous acute and chronic inflammatory processes (reviewed in Morgan (1994)). For example, cleavage of C3 generates both C3a, a relatively weak chemoattractant for neutrophils and C3b, a potent opsonin. The subsequent cleavage of C5 generates C5a, a potent chemo-attractant and activator of neutrophils, as well as C5b, which initiates deposition of the membrane attack complex C5b-9 on the cell surface. The recruitment of neutrophils contributes significantly to inflammatory tissue injury in numerous experimental systems (reviewed in Korthius and Granger (1994)), while C5b-9 deposition can result in cell activation due to calcium influx (Morgan, 1989), upregulation of cell surface adhesion molecules (Hattori *et al.*, 1989) or in cell lysis.

The complement cascade is regulated by endogenous inhibitors which act at numerous points including C1, C4, C3 convertase activity, C5 convertase activity and the formation of C5b-9 (Lachmann, 1991). Development of a soluble form of complement receptor type 1 (sCR1), an inhibitor of C3 convertase activity, has provided a means to examine the potential of complement inhibition for the amelioration of several disease processes including xenotransplant rejection (Pruitt *et al.*, 1994), lung and dermal immune complex-mediated injury (Mulligan *et al.*, 1992), experimental allergic encephalomyelitis (Piddlesden *et al.*, 1994), cardiopulmonary bypass (Moat *et al.*, 1992; Gillinov *et al.*, 1993) and reperfusion injury (Weisman *et al.*, 1990; Smith *et al.*, 1993; Pemberton *et al.*, 1993). However, inhibition of the complement cascade by sCR1 at the level of C3 convertase activity may be predicted to have significant clinical side effects. Generation of C3b is essential for the normal phagocytosis of bacterial and fungal pathogens as well as the clearance of circulating immune complexes (Liszewski and Atkinson, 1993). In fact, humans genetically deficient in C3 are subject to recurrent life-threatening infections and also suffer from a greatly increased incidence of autoimmune diseases such as systemic lupus erythrematosus and glomerulonephritis (Ross and Densen, 1984).

To avoid these complications, we have focused on the

*Author to whom correspondence should be addressed.
†Current address: Women's Health Research Institute, Wyeth-Ayerst Research, Radnor, PA 19087, U.S.A.

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inhibition of the complement cascade at the level of C5, thereby blocking formation of the proinflammatory complement activation products C5a and C5b-9, while preserving the generation of C3b. Patients with a genetic deficiency of C5 suffer only from an increased incidence of Neisserial infections, which are in fact milder than infections in patients having normal levels of C5 (Ross and Densen, 1984). As there are no endogenous inhibitors specific for the C5 convertases, monoclonal antibodies were developed, which bind human C5 and prevent its cleavage by C5 convertase (Würzner *et al.*, 1991; Khroshus *et al.*, 1995). These antibodies inhibit complement-mediated acute cardiac tissue injury occurring in perfused heart models of xenotransplantation (Kroshus *et al.*, 1995) and block leukocyte and platelet activation in an *ex vivo* recirculation model of cardiopulmonary bypass (Rinder *et al.*, 1995). Additionally, in pigs, a monoclonal antibody against C5a reduces myocardial infarct size by 35% (Amsterdam *et al.*, 1995). Finally, a monoclonal antibody against mouse C5 (Frei *et al.*, 1987) blocks progression of established arthritis in a murine collagen-induced arthritis model (Wang *et al.*, 1995) and prevents development of glomerulonephritis in a murine model of systemic lupus erythromatosis (Wang *et al.*, 1995).

The desired pharmacological properties of complement inhibitors are dramatically different for acute versus chronic settings. In acute settings, a complement inhibitor should have a relatively short serum half-life, coupled with a rapid rate of tissue penetration, since complement activation occurs within the tissue (Schäfer *et al.*, 1986; Hugo *et al.*, 1990). Large molecules such as monoclonal antibodies have very slow rates of tissue penetration as compared to smaller molecules such as Fab fragments (Covell *et al.*, 1986) or single chain Fv (scFv) molecules (Yokota *et al.*, 1992). A complement inhibitor used in chronic settings should have a prolonged serum half-life and not provoke an immune response. In humans, murine monoclonal antibodies have a half-life of approximately 1 day (LoBuglio *et al.*, 1993) and uniformly provoke an immune response (Khazaeli *et al.*, 1994). These problems have been addressed by the development of humanized monoclonal antibodies which have serum half-lives of several days (LoBuglio *et al.*, 1989; Khazaeli *et al.*, 1991; Saleh *et al.*, 1992; Schüpbach *et al.*, 1993) as well as greatly diminished immunogenicity (LoBuglio *et al.*, 1993)

In this study, variants of the 5G1.1 monoclonal antibody have been engineered, which can be used in either acute or chronic settings. Firstly, the 5G1.1 variable regions were humanized using the CDR-grafting technique (Riechmann *et al.*, 1988). The humanized variable regions were then used to construct humanized antibody, Fab and scFv molecules which all maintained a high affinity for human C5 and blocked the generation of both C5a and C5b-9.

MATERIALS AND METHODS

Cloning of 5G1.1 *variable region genes*

For N-terminal amino acid sequencing, 75 μg 5G1.1 mAb was subjected to SDS–PAGE under reducing conditions and transferred to ProBlott membrane (Applied Biosystems, Foster City, CA, U.S.A.) as previously described (Evans *et al.*, 1995). Protein bands were localized by staining with Ponceau S, excised and subjected to amino acid sequence analysis using Edman chemistry, performed on a pulsed liquid protein sequencer (ABI model 477A) with the PTH amino acids analysed using an on-line microbore HPLC system (ABI model 120A).

To deblock the amino terminus of the 5G1.1 heavy chain, 10 mg 5G1.1 monoclonal antibody was exchanged into reducing buffer (5 M guanidine–HCl, 50 mM Tris–HCl, 10 mM dithiothreitol, pH 8.5), using a PD-10 column (Pharmacia, Piscataway, NJ, U.S.A.). After incubation for 1 hr at room temperature, 50 mM iodoacetamide was added and the incubation allowed to continue for 30 min. The carbamidomethylated light and heavy chains were then separated by size exclusion chromatography on a Superose 12 (Pharmacia) column equilibrated with 5 M guanidine–HCl, 50 mM Tris–HCl (pH 8.5). The purified light chain was exchanged into 50 mM sodium phosphate (pH 7.0), using a PD-10 column, digested using 0.5 mU pyroglutamate aminopeptidase (PanVera, Madison, WI, U.S.A.) per nmol of heavy chain protein and sequenced as previously described. For determination of internal amino acid sequence, the isolated light chain was exchanged into 2 M urea, 25 mM Tris–HCl, 1 mM EDTA (pH 8.0) and incubated at 37°C overnight with endoproteinase Lys-C (Promega, Madison, WI, U.S.A.) at a protease:protein ratio of 1:40. The digested material was run on a C18 reversed phase HPLC column (Beckman Instruments, Fullerton, CA, U.S.A.) and eluted using a linear acetonitrile gradient (0–50%) in 0.1% trifluoroacetic acid. Peaks were subjected to amino acid sequence analysis as previously described.

Cloning of the 5G1.1 heavy chain variable region (VH) and light chain variable region (VL) was initially performed using a set of commercially available primers (Mouse Ig-Primer Set, catalogue number 69831-1; Novagen, Madison, WI, U.S.A.) essentially as previously described (Evans *et al.*, 1995). Comparison to the obtained peptide sequences confirmed that the correct VH had been cloned, whereas neither of the two light chain amino acid sequences were present in the cloned VL. To isolate the 5G1.1 VL, the UWGCG program TFASTA was used to search the GenBank rodent subdirectory with the amino acid sequence obtained from the purified light chain (IQMTQSPASLSASVGETVT). An exact match to this sequence was located in the murine germline gene encoding the v-kappa k2 variable region (Seidman *et al.*, 1978). The DNA sequence of this germline gene was used to design an oligonucleotide for use as a variable region 5′-primer for isolation of the correct VL gene.

Humanization of 5G1.1 *variable regions*

Two humanized variants of the 5G1.1 VL and VH regions were constructed by CDR grafting (Reichmann *et al.*, 1988). For CDR grafting, the 5G1.1 heavy and light complementarity determining regions were intro-

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duced into the human heavy variable region H20C3H (Weng *et al.*, 1992) to yield h5G1.1VHC (Fig. 1A) or the human light variable region I.23 (Klein *et al.*, 1993) to yield h5G1.1VLC (Fig. 1B), respectively. Construction of each CDR-grafted variable region was performed by PCR amplification, using two synthetic overlapping oligonucleotides of approximately 200 nucleotides in length. The cloned PCR products were sequenced on both strands in their entirety to ensure the absence of PCR-introduced errors. Murine amino acids were introduced into the human framework regions at position 71 in the light variable region and positions 71 and 78 in the heavy variable region by PCR mutagenesis.

Expression and purification of Fab

Expression of chimeric and humanized 5G1.1 Fab molecules was achieved using the mammalian 293-EBNA expression system essentially as previously described (Evans *et al.*, 1995*b*). Briefly, a plasmid capable of producing a chimeric Fab was constructed by fusion of the murine VL to the human kappa (*Inv*3 allele) constant region and fusion of the murine VH to the human IgG1 (*G1m*(17) allele) Fd in the expression plasmid pAPEX-3P. Since the cloning strategy for the 5G1.1 VL did not obtain the leader sequence, the leader sequence of the human CD59 protein was cloned in frame to allow secretion of the chimeric light chain cDNA. Similarly, a plasmid capable of expressing the CDR grafted Fab, h5G1.1 Fab (CDR), was constructed by cloning the variable regions h5G1.1VHC and h5G1.1VLC into pAPEX-3P. Finally, a plasmid capable of expressing the CDR grafted Fab containing framework changes, h5G1.1 Fab (CDR + FW), was constructed by cloning the variable regions h5G1.1VHC+F and h5G1.1VLC+F into pAPEX-3P. 293-EBNA cells (Invitrogen, San Diego, CA, U.S.A.) were transfected with the pAPEX-3P expression plasmids and selected using 1 μg/ml puromycin. Secreted Fabs were purified using protein G Sepharose chromatography as described (Evans *et al.*, 1995*b*), dialysed into PBS, and stored at 4°C. Protein concentrations were determined by the Bradford assay (Bio-Rad, Melville, NY, U.S.A.).

Construction of a humanized scFv

Two scFv proteins were constructed by the overlapping PCR technique. The h5G1.1 scFv (CDR) contained the h5G1.1VLC variable region, linked to the h5G1.1VHC variable region; while the h5G1.1 scFv (CDR + FW) contained the h5G1.1VLC+F variable region, linked to the h5G1.1VHC+F variable region. These scFv cDNAs were cloned into a plasmid in which expression is driven by the trc promoter. *Escerichia coli* strain ME1 was transformed with the resulting expression plasmids. Transformants were grown at 37°C in 2 l Applikon glass vessel fermentors containing Terrific Broth (1.2% (w/v) bactotryptone, 2.4% (w/v) bacto-yeast extract, 0.4% (v/v) glycerol, 90 mM potassium phosphate, pH 7.0), supplemented with 100 μg/ml ampicillin. The production of recombinant scFv was induced by the addition of 1 mM isopropylthio-β-D-galactoside when the OD_{550} of the culture reached 10. After an additional 3 hr incubation at 37°C, the cells were harvested by centrifugation and the cell pellets stored at −20°C. Cells were resuspended in 1 mM EDTA (pH 5.0), at 10 ml/g weight and lysed by a single pass through a microfluidizer M110-T (Microfluidics Corp., Newton, MA, U.S.A.). After centrifugation at 17 500**g** for 15 min, the resulting inclusion body pellet was resuspended in 20 mM Tris–HCl (pH 8.0), 1 mM EDTA, 100 mM NaCl, 0.15% (w/v) deoxycholate at 10 ml/g inclusion body, using a Tekmar polytron. The inclusion bodies were again pelleted by centrifugation at 17 500***g*** for 15 min and resuspended in 20 mM Tris–HCl (pH 9.0) and 8 M urea at 10 ml/g. After stirring for 1 hr, the sample was centrifuged at 14 000***g*** for 30 min to pellet remaining insoluble material. The resulting supernatant was diluted 10-fold with 20 mM Tris–HCl (pH 9.0), 7 M urea and 50 μM cupric sulfate, and allowed to stir for at least 16 hr at 4 C to refold the scFv. Biocryl BPA-1000 (TosoHaas, Montgomeryville, PA, U.S.A.) was then added as a flocculation agent at 3 μl/ml (or at 10 μl/ml for the anti-mouse C5 scFv). After stirring for 5 min, the sample was centrifuged at 15 000***g*** for 10 min to pellet insoluble material. The supernatant was exchanged into 20 mM Tris (pH 9.0), 1 mM EDTA by diafiltration and concentrated by ultrafiltration using a stirred cell, fitted with a YM10 membrane (Amicon, Beverly, MA, U.S.A.). Properly refolded scFv was separated from aggregated material and contaminating proteins by anion exchange chromatography using Q Sepharose Fast Flow (Pharmacia). Bound scFv was eluted with 20 mM Tris–HCL (pH 9.0) and 1 mM EDTA containing a linear NaCl gradient (0–0.5 M). The fractions containing the scFv were combined, concentrated by ultrafiltration using a stirred cell fitted with a YM10 membrane and applied to a Sephacryl S200 HR 26/100 column (Pharmacia) equilibrated in 20 mM Tris–HCl, pH 9.0, 1 mM EDTA and 150 mM NaCl. Fractions containing the scFv were combined, exchanged into PBS by diafiltration, concentrated by ultrafiltration, filtered through a 0.22 mm Millex-GV filter (Millipore, Bedford, MA, U.S.A.) and stored at 4°C. Protein concentration was determined by the Bradford assay.

ELISA binding assays

For detection of recombinant Fab binding to human C5, F96 PolySorp microtiter plates (Nunc, Naperville, IL, U.S.A.) were coated overnight at 4°C with 0.1 mg/well human C5 (Quidel, San Diego, CA, U.S.A.) at a concentration of 2 mg/ml in 0.1 M Na_2CO_3, pH 9.6. The plates were then washed three times with 100 μl/well wash buffer (PBS containing with 0.5% (v/v) Tween 20) and blocked with 100 μl/well blocking buffer (PBS supplemented with 1% (w/v) bovine serum albumin, fraction V and 0.5% (v/v) Tween 20) at 37°C for 1 hr. The plates were again washed three times with wash buffer and incubated with 50 μl/well blocking buffer, containing 30 ng/ml murine 5G1.1 mAb, plus the indicated concentrations of inhibitor, at 37°C for 2 hr. The plates were

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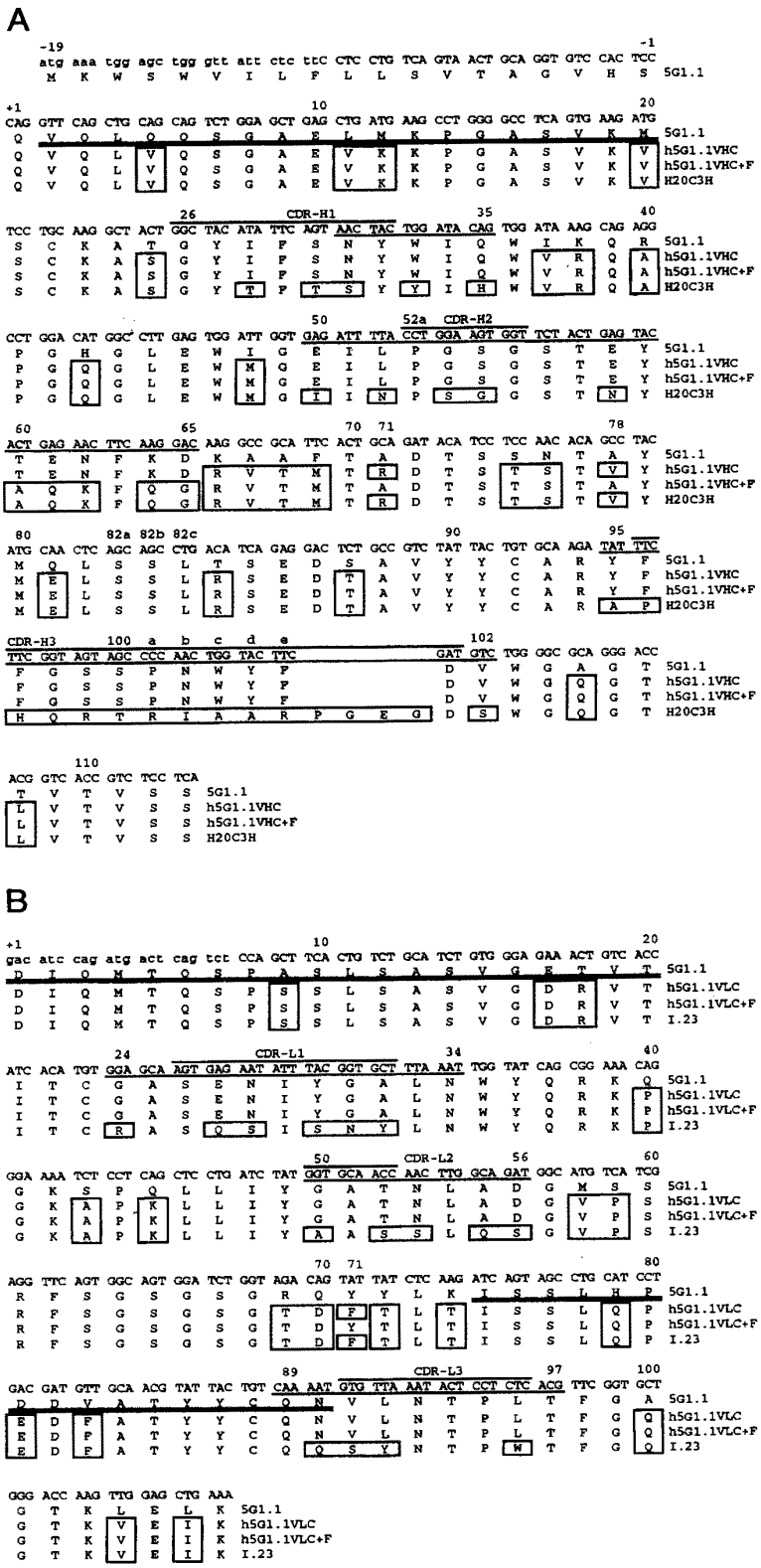
A
-19 -1
atg aaa tgg agc tgg gtt att ctc ttc CTC CTG TCA GTA ACT GCA GGT GTC CAC TCC
M K W S W V I L F L L S V T A G V H S 5G1.1
+1 10 20
CAG GTT CAG CTG CAG CAG TCT GGA GCT GAG CTG ATG AAG CCT GGG GCC TCA GTG AAG ATG
Q V Q L Q Q S G A E L M K P G A S V K M 5G1.1
Q V Q L V Q S G A E V K K P G A S V K V h5G1.1VHC
Q V Q L V Q S G A E V K K P G A S V K V h5G1.1VHC+F
Q V Q L V Q S G A E V K K P G A S V K V H20C3H
26 CDR-H1 35 40
TCC TGC AAG GCT ACT GGC TAC ATA TTC AGT AAC TAC TGG ATA CAG TGG ATA AAG CAG AGG
S C K A T G Y I F S N Y W I Q W I K Q R 5G1.1
S C K A S G Y I F S N Y W I Q W V R Q A h5G1.1VHC
S C K A S G Y I F S N Y W I Q W V R Q A h5G1.1VHC+F
S C K A S G Y T F T S Y Y I H W V R Q A H20C3H
50 52a CDR-H2
CCT GGA CAT GGC CTT GAG TGG ATT GGT GAG ATT TTA CCT GGA AGT GGT TCT ACT GAG TAC
P G H G L E W I G E I L P G S G S T E Y 5G1.1
P G Q G L E W M G E I L P G S G S T E Y h5G1.1VHC
P G Q G L E W M G E I L P G S G S T E Y h5G1.1VHC+F
P G Q G L E W M G I I N P S G G S T N Y H20C3H
60 65 70 71 78
ACT GAG AAC TTC AAG GAC AAG GCC GCA TTC ACT GCA GAT ACA TCC TCC AAC ACA GCC TAC
T E N F K D K A A F T A D T S S N T A Y 5G1.1
T E N F K D R V T M T R D T S T S T V Y h5G1.1VHC
A Q K F Q G R V T M T A D T S T S T A Y h5G1.1VHC+F
A Q K F Q G R V T M T R D T S T S T V Y H20C3H
80 82a 82b 82c 90 95
ATG CAA CTC AGC AGC CTG ACA TCA GAG GAC TCT GCC GTC TAT TAC TGT GCA AGA TAT TTC
M Q L S S L T S E D S A V Y Y C A R Y F 5G1.1
M E L S S L R S E D T A V Y Y C A R Y F h5G1.1VHC
M E L S S L R S E D T A V Y Y C A R Y F h5G1.1VHC+F
M E L S S L R S E D T A V Y Y C A R A P H20C3H
CDR-H3 100 a b c d e 102
TTC GGT AGT AGC CCC AAC TGG TAC TTC GAT GTC TGG GGC GCA GGG ACC
F G S S P N W Y F D V W G A G T 5G1.1
F G S S P N W Y F D V W G Q G T h5G1.1VHC
F G S S P N W Y F D V W G Q G T h5G1.1VHC+F
H Q R T R I A A R P G E G D S W G Q G T H20C3H
110
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T V T V S S 5G1.1
L V T V S S h5G1.1VHC
L V T V S S h5G1.1VHC+F
L V T V S S H20C3H
B
+1 10 20
gac atc cag atg act cag tct CCA GCT TCA CTG TCT GCA TCT GTG GGA GAA ACT GTC ACC
D I Q M T Q S P A S L S A S V G E T V T 5G1.1
D I Q M T Q S P S S L S A S V G D R V T h5G1.1VLC
D I Q M T Q S P S S L S A S V G D R V T h5G1.1VLC+F
D I Q M T Q S P S S L S A S V G D R V T I.23
24 CDR-L1 34 40
ATC ACA TGT GGA GCA AGT GAG AAT ATT TAC GGT GCT TTA AAT TGG TAT CAG CGG AAA CAG
I T C G A S E N I Y G A L N W Y Q R K Q 5G1.1
I T C G A S E N I Y G A L N W Y Q R K P h5G1.1VLC
I T C G A S E N I Y G A L N W Y Q R K P h5G1.1VLC+F
I T C R A S Q S I S N Y L N W Y Q R K P I.23
50 CDR-L2 56 60
GGA AAA TCT CCT CAG CTC CTG ATC TAT GGT GCA ACC AAC TTG GCA GAT GGC ATG TCA TCG
G K S P Q L L I Y G A T N L A D G M S S 5G1.1
G K A P K L L I Y G A T N L A D G V P S h5G1.1VLC
G K A P K L L I Y G A T N L A D G V P S h5G1.1VLC+F
G K A P K L L I Y A A S S L Q S G V P S I.23
70 71 80
AGG TTC AGT GGC AGT GGA TCT GGT AGA CAG TAT TAT CTC AAG ATC AGT AGC CTG CAT CCT
R F S G S G S G R Q Y Y L K I S S L H P 5G1.1
R F S G S G S G T D F T L T I S S L Q P h5G1.1VLC
R F S G S G S G T D Y T L T I S S L Q P h5G1.1VLC+F
R F S G S G S G T D F T L T I S S L Q P I.23
89 CDR-L3 97 100
GAC GAT GTT GCA ACG TAT TAC TGT CAA AAT GTG TTA AAT ACT CCT CTC ACG TTC GGT GCT
D D V A T Y Y C Q N V L N T P L T F G A 5G1.1
E D F A T Y Y C Q N V L N T P L T F G Q h5G1.1VLC
E D F A T Y Y C Q N V L N T P L T F G Q h5G1.1VLC+F
E D F A T Y Y C Q Q S Y N T P W T F G Q I.23
GGG ACC AAG TTG GAG CTG AAA
G T K L E L K 5G1.1
G T K V E I K h5G1.1VLC
G T K V E I K h5G1.1VLC+F
G T K V E I K I.23

again washed three times with wash buffer, and incubated with 50 μl/well blocking buffer, containing peroxidase-conjugated goat anti-mouse IgG Fc antibody, at a 2000-fold dilution (Sigma, St. Louis, MO, U.S.A.), at 37°C for 1 hr. After three final washes, the plate was developed with 50 μl/well substrate buffer (0.05 M phosphate–citrate buffer, pH 5.0, containing 0.3 mg/ml sodium perborate and 0.4 mg/ml *o*-phenylenediamine dihydrochloride). Reactions were stopped by the addition of 50 μl/well 1 M sulfuric acid and quantified using a Bio-Rad model 3550 plate reader set at 490 nm.

Hemolytic and C5a generation assays

The antibody, Fab, or scFv was pre-incubated with the indicated percent (v/v) human serum in 0.1 ml veronal buffered saline containing 1% gelatin ($GVBS^{++}$) for 30 min at room temperature. Chicken erythrocytes (Lampire Biological, Pipersville, PA, U.S.A.) were washed four times with $GVBS^{++}$ and resuspended at 5×10^7 cells/ml in $GVBS^{++}$, containing 1 μg/ml anti-chicken erythrocyte IgG (Inter-cell Technologies, Hopewell, NJ, U.S.A.). After incubation at 4°C for 15 min, the cells were washed twice with $GVBS^{++}$ and 3×10^6 cells were added to the pre-incubated human serum. After further incubation for 30 min at 37°C, the cells were pelleted by centrifugation at 10 000*g* for 2 min and the supernatant was assayed for released hemoglobin by measurement of the absorbance at 415 nm, and for C5a content by sandwich ELISA as described (Würzner *et al.*, 1991).

Porcine aortic endothelial cell (PAEC) lysis assays

PAEC were obtained from Cell Systems (Kirkland, WA, U.S.A.) at passage 1 and maintained in M199 medium, supplemented with 20% fetal calf serum, 20 mM HEPES, 10 μg/ml heparin (Sigma) and 5 μg/ml ECGF (Biomedical Technologies, Stoughton, MA, U.S.A.). PAECs at passage 3 were plated at 10 000 cells/well in a 96-well Falcon flat-bottomed microtiter plate. The following day, cells were washed twice with Hank's balanced salt solution, containing 1% (w/v) bovine serum albumin (HBSS/BSA) and incubated with 10 μM calcein AM (Molecular Probes, Eugene, OR, U.S.A.) in HBSS/BSA for 30 min at 37°C. Wells were washed twice with HBSS/BSA and incubated with 0.85 mg/ml rabbit anti-PAEC antibody (Kennedy *et al.*, 1994) for 30 min at 37°C. Wells were again washed twice with HBSS/BSA and incubated with HBSS containing 20% (v/v) human serum plus various concentrations of inhibitors for 30 min at 37°C. The supernatant was then transferred to a new 96-well Falcon flat-bottom microtiter plate and the remaining cells were solubilized with 1% SDS. The fluorescence of the supernatants and solubilized cells was determined using a Cytofluor 2350 (Millipore) with an excitation wavelength of 485 nm and an emmision wavelength of 530 nm. Percentage dye release was defined as the ratio of supernatant fluorescence to total (supernatant plus cell) fluorescence. Supernatant fluorescence in the absence of human serum was subtracted as background release.

RESULTS

Humanization of 5G1.1

The variable regions of the anti-C5 monoclonal antibody 5G1.1 were cloned by PCR amplification using 5′ primers, based on amino acid sequencing (for the light variable region) or degenerate 5′ primers (for the heavy variable region), coupled with 3′ primers specific for murine constant region sequences. Amino acid sequence analysis confirmed that the appropriate heavy and light variable regions had been cloned (Fig. 1). The 5G1.1 heavy variable region was most similar to V_H genes belonging to the V_H1/J558 family (Kofler *et al.*, 1992) and utilized the J_H1 gene. The most similar cloned murine V_H region was from the IgM secreting hybridoma 19.1.2 (Akolkar *et al.*, 1987). The 5G1.1 V_H differed from this clone at positions 20, 37 and 68 in framework regions, as well as at positions 28, 31 and 35 in CDR-H1, positions 58 and 65 in CDR-H2 and positions 95, 96 and 97 in CDR-H3. The 5G1.1 light variable region was most similar to the NYC V_κ gene (Jack *et al.*, 1992) and utilized the mouse $J_\kappa5$ gene (Hieter *et al.*, 1980), with amino acid differences present at framework position 72 in framework region 3 and at position 93 in CDR-L3.

For humanization, the 5G1.1 heavy variable regions CDRs were transposed into the human variable region

Fig. 1. Sequence of the 5G1.1 heavy (A) and light (B) variable regions. The DNA sequence and the translated amino acid sequence of the cloned 5G1.1 variable regions are shown. Amino acid position is numbered according to Kabat *et al.* (1992), with the complementarity determining regions according to the hypervariable sequence definition (Kabat *et al.*, 1992) or the structural variability definition (Chothia and Lesk, 1987) underlined and overlined, respectively. Lower case letters indicate nucleotide sequences derived from primers used for cloning. Amino acid sequences obtained from protein sequencing are indicated by double underlines. The protein sequences of the human variable regions H20C3H and I.23 are shown below the appropriate 5G1.1 variable regions. h5G1.1VHC and h5G1.1VLC denote humanized heavy and light variable regions constructed by grafting the CDRs from 5G1.1 on to the H20C3H and I.23 human framework regions. The variable regions h5G1.1 VHC+F and h5G1.1VLC+F contain murine amino acids at framework positions 71 and 78 in the heavy variable region and position 71 in the light variable region in addition to the murine CDR sequences. Amino acids in the human variable regions and the humanized 5G1.1 variable regions which differ from the murine 5G1.1 sequences are boxed.

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H20C3H (Weng *et al.*, 1992). This human V_H was derived from the human genomic V_H gene HG3 (Rechavi *et al.*, 1983), belonging to the V_H1 family and the human genomic J_H5 gene, and contains no changes in the framework regions from these genomic genes. The 5G1.1 light variable region CDRs were grafted into the human light variable region I.23 (Klein *et al.*, 1993). This human V_L region was derived from the human $V_\kappa I$ family gene O12 (Klein *et al.*, 1993) and the genomic $J_\kappa 1$ gene, with the introduction of an Arg residue in framework region 2 at position 38 in the I.23 cDNA as compared to the Gln residue encoded in the O12 genomic gene. Initial humanized 5G1.1 variable heavy and light regions were constructed by introducing the 5G1.1 CDRs into these human frameworks and are designated as h5G1.1 VHC and 5G1.1 VLC (Fig. 1).

Computer modeling of antibody variable regions has indicated that amino acid residues within the framework regions of a variable region can influence the structure of the CDRs. Comparison of the murine 5G1.1 variable region with the human acceptor variable regions H20C3H and I.23 suggested that the murine and human framework regions differed at three positions important for CDR structure. In the light chain, the presence of either Phe or Tyr at framework position 71 determines the canonical structure of CDR-L1 (Chothia and Lesk, 1987; Foote and Winter, 1992). Since I.23 contains a Phe residueat position 71, whereas 5G1.1 contains a Tyr residue, the Phe residue in h5G1.1 VLC was changed to a Tyr residue to create the variable region h5G1.1 VLC+F (Fig. 1). Similarly, the human H20C3H and murine 5G1.1 V_H regions differ at framework positions 71 and 78, which have been suggested to be important for maintaining the function of humanized antibodies (Carter *et al.*, 1992; Chothia *et al.*, 1989; Tramontano *et al.*, 1990; Foote and Winter, 1992). The murine amino acids were, therefore, introduced at these positions to create the variable region h5G1.1 VHC+F (Fig. 1).

Since choice of human antibody isotype can influence antibody avidity whereas isotype choice does not influence Fab affinity (Morelock *et al.*, 1994), initial analysis of the humanization procedure was performed using humanized Fab fragments. The murine 5G1.1 V_L and its humanized variants h5G1.1VLC and h5G1.1VLC+F were fused to the human kappa constant region (Inv3 allele), to create an intact light chain. The 5G1.1 V_H and its humanized variants h5G1.1VHC and h5G1.1VHC+F were fused to constant region 1 of human IgG1 (*G1m*(17) allele) to create an intact Fd. Three humanized Fabs were produced in 293-EBNA cells

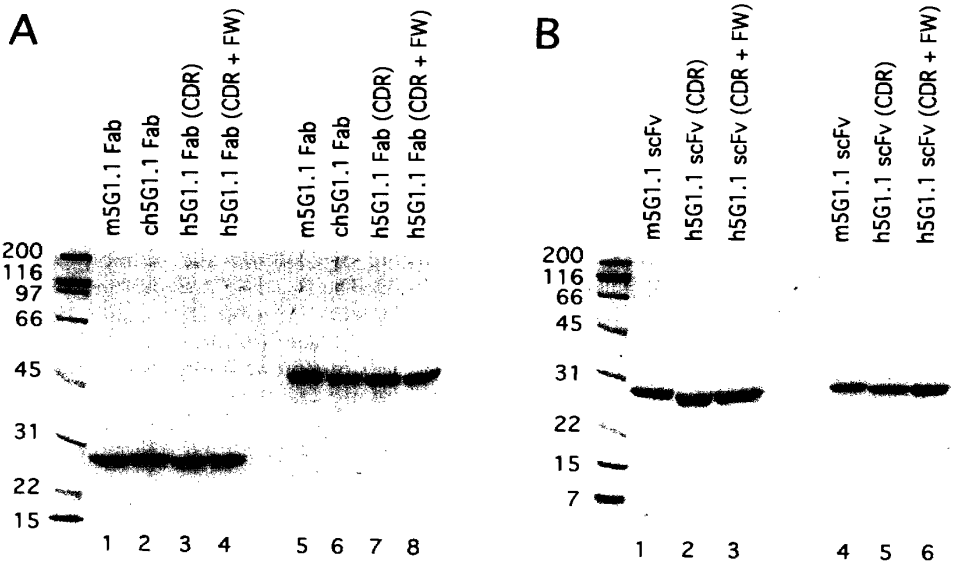

Fig. 2. SDS–PAGE analysis of purified recombinant proteins. (A) Fab fragments from the murine 5G1.1 mAb (m5G1.1 Fab) were produced by papain digestion of the m5G1.1 mAb followed by protein A chromatography to remove undigested antibody and Fc fragments. Recombinant chimeric Fab (ch5G1.1 Fab), CDR-grafted Fab (h5G1.1 Fab (CDR)), or CDR-grafted Fab containing murine framework amino acids (h5G1.1 Fab (CDR + FW)) were produced in 293 cells and purified from conditioned, serum-free medium using protein G-sepharose chromatography. Protein samples (10 mg) were subjected to SDS–PAGE under reducing (lanes 1–4) or non-reducing conditions (5–8) and stained with Coomassie R-250. (B) scFv molecules containing the murine variable regions (m5G1.1 scFv), the CDR-grafted variable regions (h5G1.1 scFv (CDR)), or the CDR-grafted variable regions containing murine framework amino acids (h5G1.1 scFv (CDR + FW)) were produced in *E. coli* and purified, as described in Materials and Methods. Ten micrograms of each purified scFv was subjected to SDS–PAGE under reducing (lanes 1–3) or non-reducing (lanes 4–6) conditions and stained with Coomassie R-250.

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by co-expression of light chain and Fd: (1) ch5G1.1 Fab, a recombinant chimeric Fab containing the murine 5G1.1 variable regions; (2) h5G1.1 Fab (CDR), a recombinant humanized Fab containing the h5G1.1VHC and h5G1.1VLC variable regions; and (3) h5G1.1 Fab (CDR + FW), a recombinant humanized Fab containing the h5G1.1VHC+F and h5G1.1VLC+F variable regions. SDS–PAGE analysis of the purified recombinant Fabs revealed a single band, which comigrated with Fab produced from 5G1.1 under non-reducing conditions and resolved into stoichiometric amounts of Fd and light chain bands, under reducing conditions (Fig. 2).

Binding of recombinant Fab and scFv to human C5

The affinities of Fab produced by papain digestion of the 5G1.1 mAb and the recombinant humanized Fabs were initially compared in a competitive binding ELISA, in which increasing concentrations of test Fab were used to displace murine 5G1.1 mAb binding to human C5 (Fig. 3). Biochemically produced Fab (m5G1.1 Fab) inhibited binding with an IC_{50} of approximately 2 nM. Similarly, the recombinant ch5G1.1 Fab, h5G1.1 Fab (CDR) and h5G1.1 (CDR+ FW) all inhibited binding by 50% at approximately 2 nM.

Although the humanized Fabs retained biological activity, neither the h5G1.1 Fab (CDR) nor the h5G1.1 Fab (CDR + FW) could be produced in significant quantities in *E. coli* (data not shown). Three scFv molecules were, therefore, constructed (Fig. 4); (1) m5G1.1 scFv, containing the murine variable light region and murine variable heavy region joined by a linker; (2) h5G1.1 scFv (CDR), containing the h5G1.1VHC and h5G1.1VLC variable regions; and (3) h5G1.1 scFv (CDR + FW), containing the h5G1.1VHC+F and h5G1.1VLC+F variable regions. These scFv molecules were produced as inclusion bodies in *E. coli*, refolded, and purified by anion exchange and sizing chromatography (Fig. 2). Gel filtration chromatography coupled with light scattering analysis demonstrated that at a concentration of 2.0 mg/ml (75 μM) the purified h5G1.1 scFv (CDR) was predominantly in the form of dimers. However, dilution of the h5G1.1 scFv resulted in a rapid shift of the equilibrium towards the monomer state (Thomas *et al.*, 1996). In the competition ELISA analysis, the m5G1.1 scFv inhibited binding of 5G1.1 mAb to human C5 with an IC_{50} value of 2 nM (Fig. 5), identical to the value obtained for the chimeric Fab (Fig. 3). Similarly, both the h5G1.1 scFv (CDR) and h5G1.1 scFv (CDR + FW) inhibited 5G1.1 mAb binding with IC_{50} values of 1.5 and 2 nM, respectively. In contrast, an scFv specific for mouse C5 (anti-mC5, Fig. 5) did not inhibit 5G1.1 binding, even at the highest concentrations assayed. Together these results indicated that the refolded scFv molecules bound to human C5 with an affinity equivalent to the Fab molecules produced in mammalian cells. Furthermore, no effect of the framework alterations could be discerned, based on the binding affinity of either the Fab or scFv molecules.

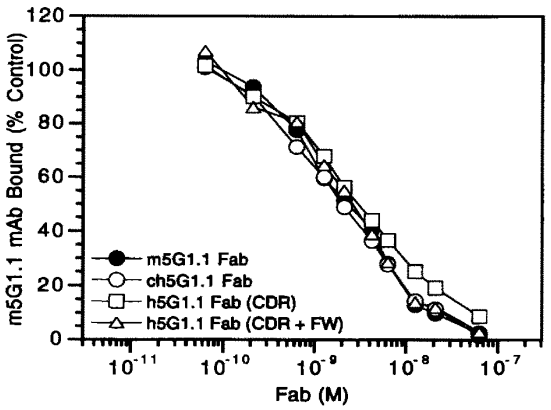

Fig. 3. Inhibition of 5G1.1 mAb binding to human C5 by recombinant Fab. 5G1.1 (0.2 nM) mAb plus the indicated concentrations of recombinant Fab were incubated together, for 2 hr at 37°C in microtiter plates coated with human C5. After washing, bound 5G1.1 mAb was detected using peroxidase-conjugated goat anti-mouse IgG (Fc specific) with quantitation performed by monitoring hydrolysis of *o*-phenylenediamine dihydrochloride at 490 nm. One hundred percent binding was defined in each experiment as the signal produced by binding of 5G1.1 mAb in the absence of competitor Fab. Data shown are mean values obtained from four to eight independent assays, with each datum point assayed in duplicate in each experiment.

Inhibition of C5b-9 formation by humanized Fab and scFv

The ability of the humanized Fab and scFv proteins to inhibit the formation of the C5b-9 membrane attack complex was evaluated using a hemolytic assay, in which the deposition of membrane attack complex on antibody-sensitized chicken erythrocytes is assessed by measuring hemoglobin release. Hemolysis was inhibited by greater than 90% when 0.25 μM of biochemically prepared murine 5G1.1 Fab, or each of the recombinant Fab molecules were added to the assay (Fig. 6A). This concentration corresponds to an approximate three-fold molar excess of Fab, as compared to the predicted human C5 concentration (0.08 μM) present in the assay. As with the Fab molecules, the scFv molecules also inhibited chicken erythrocyte hemolysis when present at a three-fold molar excess (Fig. 6B). The inhibition curves obtained with the scFv molecules were identical to those obtained with the Fab (Fig. 6B). The ability of the scFv to protect mammalian cells from complement-mediated damage was assessed by measuring their ability to block the lysis of antibody-coated porcine aortic endothelial cells (PAEC) by 20% human serum. The m5G1.1 scFv, h5G1.1 scFv (CDR) and h5G1.1 scFv (CDR + FW) all inhibited lysis of the PAEC when present at a three-fold molar excess, while the scFv specific for mouse C5 did not inhibit lysis, even at the highest concentration tested (Fig. 7A). The specificity of the 5G1.1 mAb for human C5 was maintained by the scFv, as neither the 5G1.1

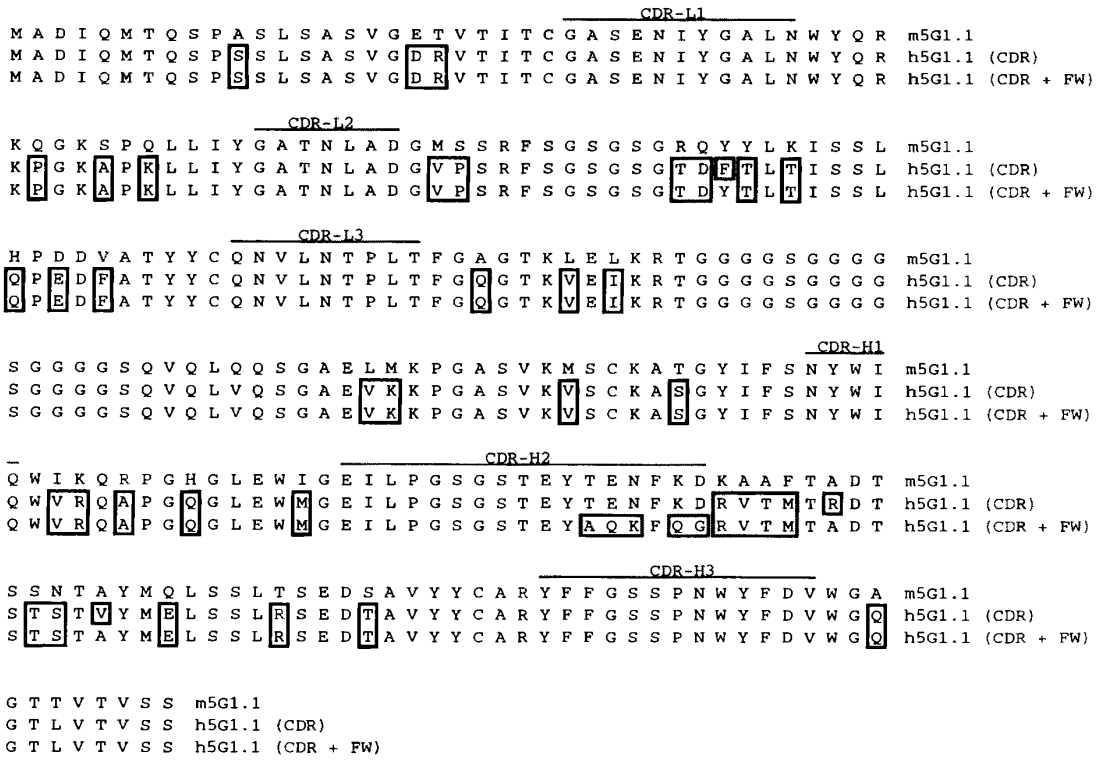

Fig. 4. Sequence of scFv molecules constructed using the 5G1.1 murine variable regions (m5G1.1) or humanized variable regions. The h5G1.1 (CDR) scFv contains the variable regions h5G1.1 VLC and h5G1.1 VHC, while the h5G1.1 scFv (CDR + FW) contains the variable regions h5G1.1 VLC+F and h5G1.1 VHC+F. The positions of the CDRs, as defined by Kabat *et al.* (1992), are indicated. Amino acid sequence analysis indicated removal of the initiator methionine, with the alanine residue at position two being the amino terminal residue of the bacterially produced scFv molecules. Amino acids in the humanized 5G1.1 variable regions which differ from the murine 5G1.1 sequences are boxed.

scFv, the h5G1.1 scFv (CDR) nor the h5G1.1 scFv (CDR + FW) could inhibit the lysis of PAEC by rat serum (Fig. 7B).

A critical parameter for use of an scFv as a C5 inhibitor is retention of the ability to block C5a generation. Previously, it has been found that a chimeric Fab, constructed from the monoclonal antibody N19-8 retained the ability to block both the formation of C5b-9 and generation of C5a, whereas an scFv constructed using the N19-8 variable regions retained the ability to block the formation of C5b-9, but only weakly inhibited the generation of C5a (Evans *et al.*, 1995). In contrast, analysis of supernatants obtained from the chicken erythrocyte hemolytic assays for C5a content (Fig. 8) indicated that equivalent concentrations of the humanized 5G1.1 Fab or scFv molecules blocked the generation of C5a. The concentration of inhibitor required for inhibition of C5a generation (Fig. 8) was comparable to the concentration of inhibitor required for inhibition of lysis (Fig. 6B).

Constuction of a humanized h5G1.1 antibody

Having demonstrated the effective humanization of the 5G1.1 variable regions, an intact humanized antibody (IgG4 isotype) was constructed and produced in 293-EBNA cells. The avidity of this humanized antibody (h5G1.1 HuG4) for human C5, was compared to the murine 5G1.1 mAb by determining the ability of each to compete binding of biotinylated 5G1.1 mAb to C5 (Fig. 9). The humanized h5G1.1 mAb had a two-fold lower avidity than the murine antibody. However, the humanized h5G1.1 HuG4 antibody was equipotent with the murine antibody at protecting PAEC from lysis by human serum, with a 0.5-fold molar ratio of antibody to C5 (1:1 ratio of antibody binding sites to C5) completely inhibiting lysis of the PAEC (Fig. 10).

DISCUSSION

This article describes the successful humanization of the anti-human C5 specific monoclonal antibody 5G1.1, utilizing the CDR-grafting technique. Although the original description of the CDR-grafting technique described the maintenance of antibody specificity by the transfer of the CDRs alone (Riechmann *et al.*, 1988), in most cases the transfer of a number of murine framework amino acids in addition to the CDRs has been found to be

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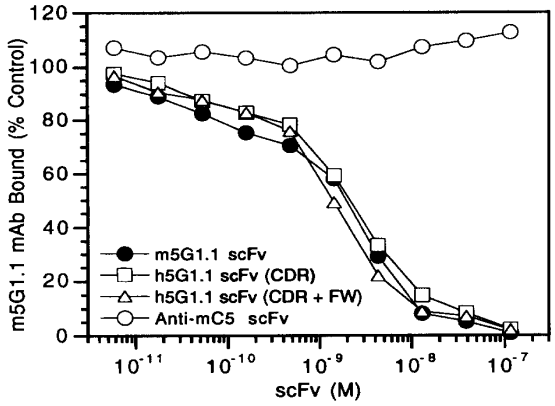

Fig. 5. Inhibition of 5G1.1 mAb binding to human C5 by recombinant scFv. 5G1.1 mAb (0.2 nM) plus the indicated concentrations of recombinant scFv were incubated together for 2 hr at 37°C, in microtiter plates coated directly with human C5. After washing, bound 5G1.1 mAb was detected using peroxidase-conjugated goat anti-mouse IgG (Fc specific) with quantitation performed by monitoring hydrolysis of *o*-phenylenediamine dihydrochloride at 490 nm. One hundred percent binding was defined in each experiment as the signal produced by binding of 5G1.1 mAb in the absence of competitor scFv. Data shown are mean values obtained from four to 10 independent assays, with each datum point assayed in duplicate in each experiment.

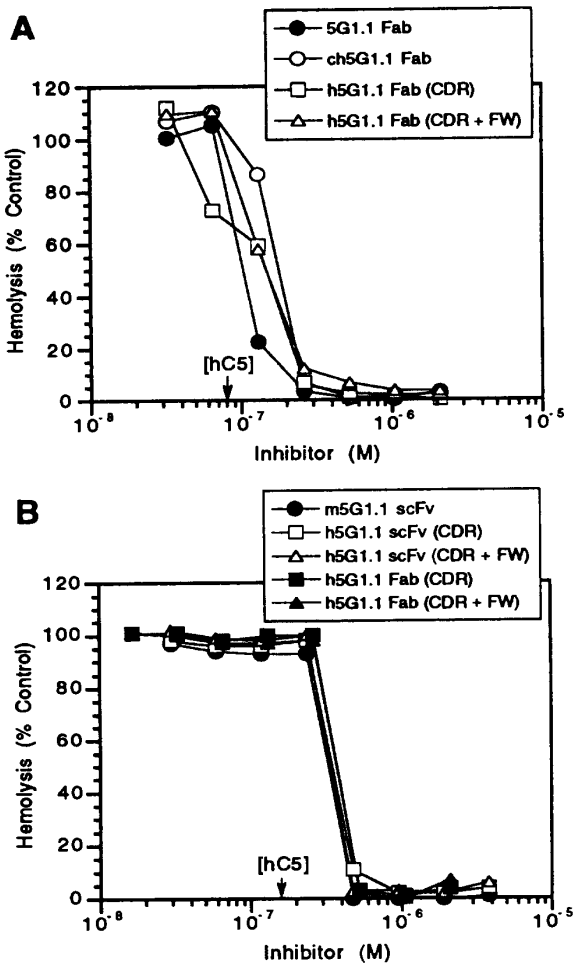

Fig. 6. Inhibition of chicken erythrocyte lysis by recombinant Fab and scFv. Twenty percent (A), or 40% (B), human serum in $GVBS^{++}$ was pre-incubated for 30 min at room temperature, with the indicated concentrations of competitors. Chicken erythrocytes, precoated with rabbit anti-chicken erythrocyte antibody, were added and the incubation was continued for 30 min at 37°C. The erythrocytes were then pelleted and the supernatants assayed for released hemoglobin by spectrophotometry (A_{415}). The A_{415} absorbance value obtained in the absence of inhibitor was defined as 100% hemolysis. Data shown are the average results of two to four independent determinations. The predicted hC5 concentrations in 20% and 40% serum are indicated by arrows.

necessary for retention of high affinity binding to the antigen (Winter and Milstein, 1991). These framework amino acids are believed to influence the structure of the CDRs. In particular, heavy variable region framework amino acid 71 determines the canonical structure of CDR-H2 (Tramontano *et al.*, 1990), while light variable region framework amino acid 71 determines the canonical structure of CDR-L1 (Chothia and Lesk, 1987; Foote and Winter, 1992). The human variable regions chosen for grafting of the 5G1.1 CDRs differed from the murine variable regions at heavy region framework position 71 and light region framework position 71. Therefore, humanized variable regions were constructed and designated h5G1.1 (CDR + FW), which maintained the murine amino acids at both of these positions.

The importance of these framework amino acids on the humanization process was initially assessed using recombinant Fab produced in 293 cells. Both the Fab containing the framework changes, h5G1.1 Fab (CDR + FW) and the Fab with unmodified human framework regions, h5G1.1 Fab (CDR), had the same binding affinity as murine Fab, when assayed by competition ELISA (Fig. 3). Similarly, no difference could be discerned between h5G1.1 Fab (CDR), h5G1.1 Fab (CDR + FW) and the murine Fab in their ability to inhibit human complement-mediated lysis of chicken erythrocytes (Fig. 6A). The h5G1.1 Fab (CDR) and h5G1.1 Fab (CDR + FW) were also equivalent in their ability to block the generation of C5a (Fig. 8). Similarly, bacterial-produced, refolded scFv, containing either human (h5G1.1 scFv (CDR)) or murine (h5G1.1 scFv (CDR + FW)) amino acids at these framework positions, had identical affinities as measured by ELISA (Fig. 5) and inhibited chicken erythrocyte lysis (Fig. 6), porcine aortic endothelial cell lysis (Fig. 7) and C5a generation (Fig. 8) with equivalent potencies. Therefore, although computer modeling suggests the importance of these framework amino acids in CDR canonical structure, amino acid changes at these positions had no effect on the functional activity of the humanized 5G1.1 molecules.

A clinically useful C5 inhibitor must block generation of both C5a and C5b-9, as both of these molecules have

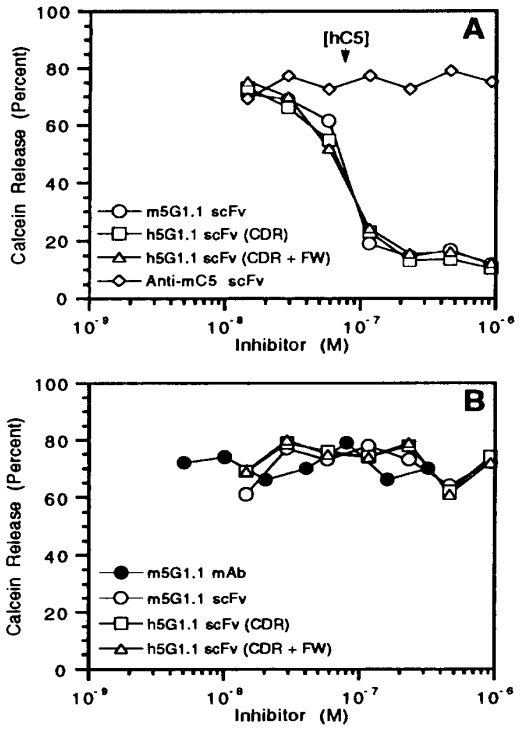

Fig. 7. Protection of porcine aortic endothelial cells (PAEC) from human serum complement by scFv. Calcein-labeled PAEC were incubated with HBSS containing 1% BSA and 0.85 mg/ml purified rabbit anti-PAEC polyclonal antibody for 30 min at 37°C. The cells were washed and incubated with HBSS containing 1% BSA plus 20% human serum (A), or 20% rat serum (B), and the indicated concentrations of inhibitor for 30 min at 37°C. Calcein dye release and retention were determined by measurement of the fluorescence of supernatants and SDS-solubilized cells, respectively, with dye release in the absence of human serum subtracted from all values. Results shown are the average of four to five independent determinations. The arrow denotes the predicted concentration of hC5 in 20% serum.

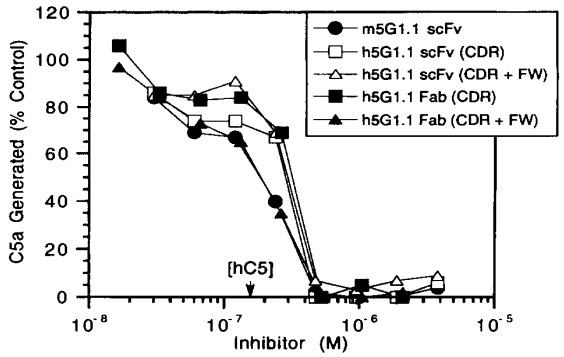

Fig. 8. Inhibition of C5a generation by recombinant Fab and scFv. Supernatants from chicken erythrocyte hemolytic assays performed using 40% human serum, as described in Fig. 5, were assayed for C5a content by ELISA. The C5a concentration obtained in the absence of inhibitor was defined as 100%. Data shown are from a representative experiment of several performed. The predicted hC5 concentration in 40% serum is indicated by the arrow.

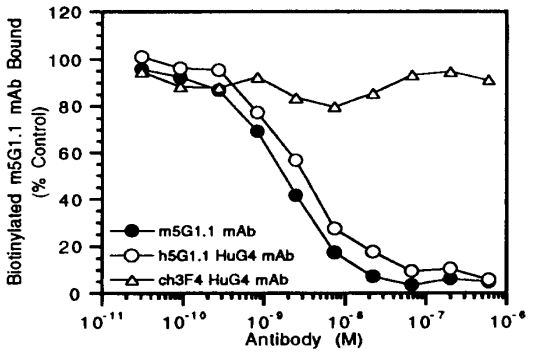

Fig. 9. Inhibition of 5G1.1 mAb binding to human C5 by recombinant humanized h5G1.1 HuG4 mAb. Biotinylated 5G1.1 mAb (0.2 nM), plus the indicated concentrations of unlabeled murine 5G1.1 mAb, recombinant h5G1.1 HuG4 mAb or a negative control recombinant HuG4 mAb (ch3F4 HuG4 mAb), were incubated together for 2 hr at 37°C in microtiter plates coated with human C5. After washing, bound biotinylated 5G1.1 mAb was detected using peroxidase-conjugated streptavidin, with quantitation performed by monitoring hydrolysis of *o*-phenylenediamine dihydrochloride at 490 nm. Absorbance values obtained in the presence of recombinant inhibitor were normalized in each individual experiment, to the value obtained in the absence of competitor. Results shown are mean values of four determinations.

potent pro-inflammatory effects. An scFv has been previously constructed from the variable regions of the monoclonal antibody N19-8 (Würzner *et al.*, 1991). The N19-8 antibody binds to human C5 with an avidity comparable to 5G1.1 (data not shown) and blocks generation of both C5b-9 and C5a. Although the N19-8 scFv has a high binding affinity for human C5 and inhibits the ability of human serum to lyse chicken erythrocytes, it only weakly inhibits C5a generation (Evans *et al.*, 1995). These findings suggest a model in which binding of the N19-8 mAb sterically blocks interaction of C5 with the C5 convertases. The N19-8 scFv may be too small to block cleavage by the C5 convertases, but remains bound to C5b and blocks formation of the C5b-9 complex. In contrast, the h5G1.1 scFv completely blocked generation of C5a when present at only a three-fold molar excess, relative to human C5 (Fig. 8). The 5G1.1 epitope may, therefore, be more directly involved in the interaction of C5 with the C5 convertases, than is the N19-8 epitope. Additionally, the h5G1.1 scFv maintains the ability to block the induction of both C5a- and C5b-dependent pro-inflammatory events in a closed loop model of cardiopulmonary bypass (S. A. Rollins, unpublished results).

Three important attributes of the humanized h5G1.1 scFv suggests its value as a therapeutic. Firstly, it main-

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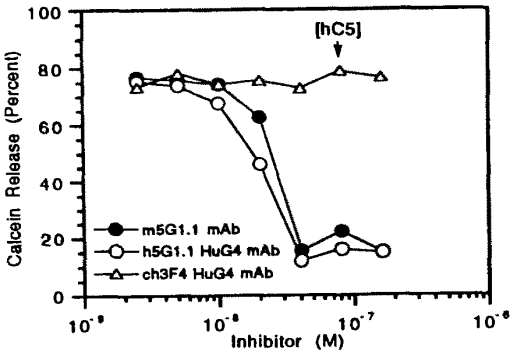

Fig. 10. Protection of porcine aortic endothelial cells from human serum complement by h5G1.1 HuG4 mAb. Calcein-labeled PAEC were incubated with HBSS containing 1% BSA and 0.85 mg/ml purified rabbit anti-PAEC polyclonal antibody for 30 min at 37°C. The cells were washed and incubated with HBSS containing 1% BSA plus 20% human serum and the indicated concentrations of inhibitor for 30 min at 37°C. Calcein dye release and retention were determined by measurement of the fluorescence of supernatants and SDS-solubilized cells, respectively, with dye release in the absence of human serum subtracted from all values. Results shown are the mean of three independent determinations. The arrow denotes the predicted concentration of hC5 in 20% serum.

tains the essential ability to block cleavage of C5 while preserving the immunoprotective properties associated with C3b generation. Secondly, it can be readily produced in large quantities in *E. coli*. Finally, the pharmacokinetics of scFv molecules are well matched to the time frame necessary for complement inhibition in acute settings where complement activation contributes significantly to the inflammatory process, such as cardiopulmonary bypass or myocardial infarction. Clearance of scFv molecules from the blood has previously been found to be extremely rapid with half lives of only a few minutes (Colcher *et al.*, 1990; Milenic *et al.*, 1991). At least two features of scFv molecules are likely to result in a rapid clearance rate, as compared to intact antibodies. Firstly, the prolonged plasma half-life of IgG is mediated through binding of the Fc region to specific "protection receptors", which have recently been demonstrated to be identical to the neonatal intestinal transport receptor (Ghetie *et al.*, 1996; Junghans and Anderson, 1996). Secondly, the small size of the scFv is well below the M_r 60 000 cut off of the glomerular basement membrane, resulting in the loss of the scFv into the urine. However, the binding of the scFv to C5 in the blood results in its incorporation into a high molecular weight scFv–C5 complex of nearly 220 000. Loss of bound scFv through glomerular filtration is, thus, dependent on the dissociation rate of the scFv from C5. Calculations based on the competitive ELISA data (Fig. 5), as well as direct measurements of h5G1.1 scFv (CDR) binding to human C5 by surface plasmon resonance (data not shown), indicated a very high affinity of the scFv for C5 ($K_d = 100$ pM) and a slow dissociation rate ($k_{off} = 1.0 \times 10^{-4}$/sec). This dissociation rate corresponds to an scFv–C5 complex half-life of approximately 2 hr. The 5G1.1 monoclonal antibody recognizes only human C5, precluding a direct assessment of h5G1.1 scFv pharmacokinetics in primates. In contrast, the N19-8 monoclonal antibody, which binds to human C5 with an avidity equivalent to 5G1.1, does bind to rhesus monkey C5. A single 100 mg bolus injection of N19-8 scFv into rhesus monkeys inhibits serum hemolytic activity for approximately 2 hr (Evans *et al.*, 1995b), further suggesting that the h5G1.1 scFv will have *in vivo* kinetic properties sufficient for use in acute clinical settings.

Complement activation has been suggested to be involved in the pathogenesis of several chronic human diseases including allograft rejection (reviewed by Baldwin *et al.* (1995)), systemic lupus erythematosus (reviewed by Mills (1994)), myasthenia gravis (reviewed by Drachman (1994)) and rheumatoid arthritis (reviewed by Morgan (1990)). Recently, it has been shown that prolonged treatment with an antibody to mouse C5 both blocks onset and disease progression in a collagen-induced arthritis model (Wang *et al.*, 1995), as well as inhibits development of glomerulonephritis in NZB/W mice (Wang *et al.*, 1996). As the half-life of the h5G1.1 scFv is likely to be too short to allow for chronic use in humans, a full length humanized antibody was also constructed. The human IgG4 isotype was chosen, as this isotype does not activate human complement (Tao *et al.*, 1993) and there is only one known allotype of IgG4 (Ghanem *et al.*, 1988), precluding the potential development of allo-antibodies in patients. The humanized h5G1.1 (CDR) HuG4 antibody bound to human C5 with a similar avidity as the murine antibody when assayed by ELISA (Fig. 9) and inhibited lysis of porcine aortic endothelial cells as effectively as the murine antibody, with a 1:1 molar ratio of antibody binding sites to human C5 being sufficient for inhibition (Fig. 10). Little information is available on the immunogenicity of CDR-grafted antibodies in humans. Repeated administration of chimeric antibodies containing intact murine variable regions, has induced an immune response directed against the murine variable regions in nearly all trials (reviewed in Khazaeli *et al.* (1994)). In this regard, it is significant that introduction of murine amino acids in the framework regions was not essential for maintenance of high affinity binding to C5. The h5G1.1 antibody is, therefore, likely to be minimally immunogenic in patients.

The recent successes of complement inhibitors in modulating numerous *in vivo* models of inflammation, suggest a great potential for complement inhibitors in the treatment of human inflammatory disease. The development of distinct C5-specific complement inhibitors with pharmacokinetic parameters, tailored for use in either acute or chronic settings, should facilitate the clinical assessment of the role of complement in such diseases.

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Examiner Name	J. L. Rogers
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See attached certification statement.

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

☒ A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Jill Gorny Sloper/	Date (YYYY-MM-DD)	2016-10-21
Name/Print	Jill Gorny Sloper, Esq.	Registration Number	60,760

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

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15260888	BELL ET AL.
	Examiner	Art Unit
	JAMES ROGERS	1644

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
STN: DISSABS MEETINGS BIOSCI CAPLUS MEDICINE	10/19/2016	JR
EAST: PGPUB, USPAT, JPO, EPO, INVENTOR SEARCH	10/19/2016	JR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
	USPAT, USPAD, SEQ ID NO: 2, 4	10/19/2016	JR

/JAMES ROGERS/ Examiner.Art Unit 1644	

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	0	DIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQKPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNVLNTPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	US-PGPUB, USPAT, USOCR, FPRS, EPO, JPO, DERWENT, IBM_TDB	OR	ON	2016/11/14 09:39

EAST Search History (Interference)

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11/14/2016 9:39:47 AM

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Dated: November 4, 2016
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888

Confirmation No.: 2558

Filed: September 9, 2016

Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (SIDS)

Dear Sir:

Applicant provides herewith a copy of an Office Action from an Application previously made of record:

Office Action (copy enclosed)				
Examiner's Initials	Serial No.:	Date Mailed from USPTO	Atty. Docket No.	Examiner
	15/284,015	11-01-2016	AXJ-114BUSCN4	J. Rogers

/JAMES L ROGERS/ 11/23/2016

The Examiner is requested to review the file history of this application, including cited references, Office Actions, Responses, etc., and is asked to contact Applicant's Attorney if the Examiner would like the Applicant to supply copies of any or all of the information included in this application. If Applicant's Attorney is not contacted by the Examiner with such a request, then it will be concluded that the Examiner has reviewed or will review the file content of this application.

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Applicant respectfully requests that the Examiner initial the blank column next to the cited Office Action, to indicate that the information has been considered by the Examiner. Alternatively, Applicant requests that the Examiner insert the phrase, "All references considered except where lined through," on each page of the Information Disclosure Statement, along with the Examiner's initials.

Pursuant to 37 C.F.R. § 1.97(e)(2), no item of information contained in this Information Disclosure Statement was cited in a communication from a foreign patent office in a counterpart foreign application and, to my knowledge after making reasonable inquiry, no item of information contained in this Information Disclosure Statement was known to any individual designated in 37 C.F.R. § 1.56(c) more than three months prior to the filing of this Information Disclosure Statement.

In accordance with 37 C.F.R. § 1.704(d)(1), the item of information contained in this Information Disclosure Statement is a communication that was issued by the United States Patent and Trademark Office, and this communication was not received by any individual designated in § 1.56(c) more than thirty days prior to the filing of this Information Disclosure Statement.

The filing of this Information Disclosure Statement is not to be interpreted as a representation that the cited document is material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any document herein be construed *per se* as a representation that such document is prior art. Moreover, Applicant understands the Examiner will make an independent evaluation of the cited document.

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

This Information Disclosure Statement is filed after the mailing date of a first Office Action on the merits, but before the mailing date of any of a Final Action under 37 C.F.R.§ 1.113, a Notice of Allowance under 37 C.F.R. § 1.311 or an action that otherwise closes prosecution in this application (37 C.F.R. § 1.97(c)). Accordingly, no fees are due with this submission (37 CFR § 1.97(b)(3)). However, the Director is hereby authorized to charge any fee deficiency which should have been filed herewith to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3.

Dated: November 4, 2016

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

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

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15260888	CTNF	12/22/2016	AXJ-114BUSCN3
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3

CONFIRMATION NO. 2558

133485
Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

PUBLICATION NOTICE

OC00000088177142

Title:TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Publication No.US-2016-0376355-A1
Publication Date:12/29/2016

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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.

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Application	Document	Mailroom Date	Attorney Docket No.
15260888	NTC.PUB	12/29/2016	AXJ-114BUSCN3

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Dated: January 19, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888 Confirmation No.: 2558

Filed: September 9, 2016 Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.111

Dear Sir:

In response to the Office Action dated December 22, 2016 (Paper No. 20161123), please amend the above-identified U.S. patent application as follows:

A Listing of the Claims is reflected on page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

LISTING OF THE CLAIMS

1. **(Original)** A method of treating a patient suffering from paroxysmal nocturnal hemoglobinuria (PNH) comprising administering to the patient a pharmaceutical composition comprising an antibody that binds C5, wherein the antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

2. **(Original)** The method of claim 1, wherein the pharmaceutical composition is administered by intravenous infusion.

3. **(Original)** The method of claim 1, wherein the antibody is administered to the patient at a dosage level of between 5 mg per kg and 50 mg per kg per patient per treatment.

4. **(Original)** The method of claim 1, wherein the pharmaceutical composition is in a single unit dosage form.

5. **(Original)** The method of claim 4, wherein the single unit dosage form is a 300 mg single unit dosage form.

6. **(Original)** The method of claim 1, wherein the pharmaceutical composition comprises a 300 mg single-use formulation of 30 ml of a 10 mg/ml sterile, preservative free solution.

7. **(Original)** The method of claim 1, wherein the patient is anemic.

8. **(Original)** The method of claim 1, wherein the patient is dosed as follows: 600 mg of the antibody via intravenous infusion every 7±1 days for 4 doses; followed by 900 mg of the antibody via intravenous infusion 7±1 days later; followed by a maintenance dose of 900 mg of the antibody via intravenous infusion every 14±2 days.

Samsung Bioepis Exhibit No. 1002
Page 1077

9. **(Original)** The method of claim 1, wherein administration of the antibody results in an immediate and sustained decrease in mean levels of lactate dehydrogenase (LDH).

10. **(Original)** The method of claim 9, wherein the immediate decrease occurs within one week of administration of the antibody.

Samsung Bioepis Exhibit No. 1002
Page 1078

REMARKS

Claims 1-10 are pending. Applicants reserve the right to pursue claims to any subject matter which they are entitled to claim in this or a separate application.

Information Disclosure Statement

Applicants acknowledge with appreciation the Examiner's indication that the Information Disclosure Statements submitted on October 21, 2016 and November 4, 2016 have been considered.

Rejection Under 35 U.S.C. § 102

Claims 1-2 and 7-10 are rejected under 35 U.S.C. § 102(b) as being anticipated by Hillmen *et al.* (*N. Engl. J. Med.* 2004; 350:552-9), as evidenced by the specification as filed (page 44) and Thomas *et al.* (Molecular Immunology, 33(17/18), pp. 1389, 1996). Specifically, the Examiner asserts that "the currently recited SEQ ID NO:2 and SEQ ID NO:4 which constitute the L and H chains of 'eculizumab' are considered to be inherent properties of the 'eculizumab' anti-C5 humanized antibody taught by Hillmen." The Examiner further states:

> [t]o the extent that applicant intends to argue that Hillmen on its own does not constitute an enabling reference because it does not explicitly place the eculizumab antibody in the public domain by not enabling one of ordinary skill in the art to make and use eculizumab comprising SEQ ID NO:2 and 4 as currently claimed, Hillmen discloses (p. 553, 3rd ¶) that eculizumab is a recombinant human antibody that binds to C5, and refers to reference number 15 (Thomas) as disclosing more information about eculizumab. Thomas in turn discloses that eculizumab is the humanized antibody h5G1.1. The '245 patent [US 6,355,245] further discloses and claims the antibody made by hybridoma 5G1.1 (see for example claims 20-21) and thus places the 5G1.1 hybridoma and the antibody produced by it into the public domain.

Applicants respectfully traverse the rejection. As described in the enclosed Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Leonard Bell (hereinafter "Declaration"), prior to the March 15, 2007 effective filing date of the present application, the complete structure of eculizumab was not disclosed in the prior art; nor was it available to the public. Specifically, Hillmen *et al.* fails to teach any part of the sequence of eculizumab, and U.S. Patent 6,355,245 (the '245 patent) fails to teach or in any way suggest the unique, non-naturally occurring, protein-

engineered full heavy chain of eculizumab (including the CH1-hinge-CH2-CH3 regions), which confers important, unexpected functional effects.

Nor does Thomas *et al.* describe eculizumab. Although Thomas *et al.* describes an antibody comprising the humanized variable region of eculizumab, the authors do not disclose, teach or suggest the full unique, protein-engineered heavy chain of eculizumab. The antibody described by Thomas *et al.* contains a *naturally-occurring* IgG4 heavy chain and is therefore not eculizumab, but rather another antibody that is structurally and functionally different from eculizumab.

Specifically, as evidenced by the sequence alignment within the Declaration, the heavy chain constant regions of eculizumab and the antibody described by Thomas *et al.* differ in fourteen amino acid residues (*i.e.*, four different residues in the CH1 domain, six different residues in the hinge region, and four different residues in the CH2 domain). Moreover, as discussed in the accompanying Declaration, these structural differences confer eculizumab with advantageous functional features over the antibody described by Thomas *et al.*, including decreased effector function, reduced immunogenicity and increased half-life.

In sum, the complete structure of eculizumab was not disclosed prior to the March 15, 2007 effective filing date of the present application. Moreover, as described in the accompanying Declaration, eculizumab was not publicly available prior to March 15, 2007. Therefore, prior to the effective filing date of the present application, the complete structure of eculizumab was neither taught nor suggested in the cited prior art; nor was it obtainable by way of public availability.

Accordingly, for at the least the above-discussed reasons, claims 1-2 and 7-10 are patentable, and the Examiner is respectfully requested to reconsider and withdraw this rejection.

Rejections Under 35 U.S.C. § 103

Claims 1-4 and 7-10 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hillmen *et al.* in view of Thomas *et al.* and Evans *et al.* (U.S. Patent No. 6,355,245). Claims 1-10 are further rejected under 35 U.S.C. § 103(a) as being unpatentable over Hillmen *et al.* in view of Thomas *et al.* and Evans *et al.*, as applied to claims 1-4 and 7-10, and in further view of Wang (U.S. Patent Publication No. 2005/0271660). The Examiner relies on Hillmen *et al.*,

Thomas *et al.* and Evans *et al.* for the reasons discussed above. The Examiner relies on Wang for teaching that eculizumab is highly soluble and that pharmaceutically acceptable carriers (*e.g.*, pyrogen-free water) can be used in formulating the antibody.

Applicants respectfully traverse these rejections. As discussed above, none of Hillmen *et al.*, Thomas *et al.* or Evans *et al.* teaches the complete structure of eculizumab. Importantly, none of these references even suggests the unique non-naturally occurring, protein-engineered heavy chain of eculizumab, which confers important, unexpected functional effects.

Wang fails to cure this deficiency. Indeed, Wang fails to teach any part of the sequence of eculizumab, let alone its unique heavy chain.

Accordingly, for at the least the above-discussed reasons, claims 1-10 are patentable, and the Examiner is respectfully requested to reconsider and withdraw this rejection.

CONCLUSION

In view of the foregoing, entry of the amendments and remarks herein, reconsideration and withdrawal of all rejections, and allowance of the instant application with all pending claims is respectfully solicited. If a telephone conversation with the Applicants` attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call the Applicants` attorney at (617) 217-4630.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3.

Dated: January 19, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

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Dated: January 19, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888

Confirmation No.: 2558

Filed: September 9, 2016

Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 CFR 1.132 BY DR. LEONARD BELL

I, Leonard Bell, M.D., a co-inventor of the subject invention, declare that:

1. I founded Alexion Pharmaceuticals, Inc. and served as its Chief Executive Officer from 1992 until 2015. In that role, I was closely involved in the development of eculizumab and in the administration and monitoring of all clinical trials involving eculizumab.

2. Prior to March 15, 2007, which is the date the subject patent application was filed, eculizumab was not available to the public. In order to be approved for marketing, eculizumab was used in clinical trials before March 15, 2007, but only under strict control and conditions of confidentiality. Patients in clinical trials of eculizumab received intravenous infusions of eculizumab at hospitals and infusion clinics and never themselves possessed samples of eculizumab. Researchers and staff involved in clinical trials of eculizumab were not permitted to use or distribute eculizumab for

purposes other than conducting the clinical trials, and were strictly accountable to Alexion for the inventory of eculizumab and for information about eculizumab.

3. Moreover, prior to March 15, 2007, the complete structure of eculizumab was not disclosed to the public. For example, the Hillmen reference (Hillmen et al., N. ENGL. J. MED., 350, pp. 552-59 (2004)) does not disclose any parts of the sequence of eculizumab and the '245 patent (U.S. Pat. No. 6,355,245) does not disclose or even hint at the unique non-naturally occurring, protein-engineered heavy chain (including the CH1-hinge-CH2-CH3 regions) of eculizumab.

4. Nor does the Thomas reference (Thomas et al., MOLECULAR IMMUNOLOGY, 33 (17/18), pp. 1389-1401 (1996)) describe eculizumab. The Thomas reference describes an antibody comprising the humanized variable region of eculizumab, but does not disclose or hint at the unique non-naturally occurring, protein-engineered heavy chain of eculizumab, which confers important functional effects (as discussed below). Rather, the antibody described in the Thomas reference contains a *naturally-occurring* IgG4 heavy chain. The antibody described in the Thomas reference therefore is not eculizumab. It is both structurally and functionally different from eculizumab.

5. As shown below, a sequence alignment of the heavy chain constant regions and hinge of eculizumab (shown in bold on the top line below) and that of the antibody described in the Thomas reference (shown on the bottom line below) demonstrates that the two antibodies are structurally very different, with fourteen different amino acid residues in the constant regions (underlined and highlighted) (four different residues in the CH1 domain, six different residues in the hinge region and four different residues in the CH2 domain):

```
----------------------------CH1-----------------------
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
```

```
----------CH1-----------------------------|Hinge-
FPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
FPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGP

-Hinge--|----------------CH2-------------------
ECPPCPAPP-VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
PCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP

---------CH2-------------------------------------
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKC
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKC

--------CH2-------|-------------CH3---------------
KVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKG
KVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKG

-------------------------CH3---------------------
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNV
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNV

---------CH3-------------
FSCSVMHEALHNHYTQKSLSLSLGK
FSCSVMHEALHNHYTQKSLSLSLGK
```

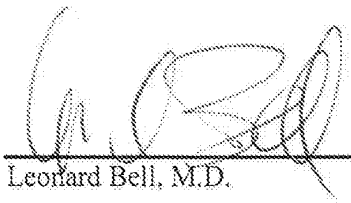
6. Also, the functional properties of eculizumab and the antibody described in the Thomas reference are very different. For example, eculizumab does not bind as strongly to Fcγ receptors as the Thomas antibody. Binding to the Fcγ receptors initiates and amplifies immune responses that are undesirable for an antibody intended to shut down the terminal complement pathway. Thus, engineering eculizumab to minimize binding to these receptors provided important advantages over the antibody described in the Thomas reference.

7. Eculizumab also has a much longer plasma half-life than the antibody described in the Thomas reference. It is advantageous for an antibody like eculizumab to remain in the circulation longer so that it does not need to be administered to patients as frequently.

8. Accordingly, as demonstrated by comparative data between eculizumab and the antibody described in the Thomas reference, the non-natural, protein-engineered, heavy chain of eculizumab reduces effector function activation, minimizes immunogenicity, and contributes to a longer half-life of eculizumab compared to the antibody described in the Thomas reference. These properties were surprising and unpredictable, and made eculizumab a better drug candidate than the antibody described in the Thomas reference.

9. In sum: the complete structure of eculizumab was not available to the public on or before March 15, 2007; eculizumab was not available to the public or being used by the public prior to that time; the unique protein-engineered heavy chain of eculizumab was not described in the Hillmen reference, the Thomas reference or the '245 patent; and the unique protein-engineered heavy chain of eculizumab could not be derived from the combined disclosures of the Hillmen reference, the Thomas reference and/or the '245 patent.

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 Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or of any patent issued thereon.



Leonard Bell, M.D. 1/18/2017 Date

Electronic Acknowledgement Receipt

EFS ID:	28107744
Application Number:	15260888
International Application Number:	
Confirmation Number:	2558
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3
Receipt Date:	19-JAN-2017
Filing Date:	09-SEP-2016
Time Stamp:	16:07:39
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		Response.pdf	150489 a0d98da3ac2ef6e19de75390ebb9b0b50a450e88	yes	7

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Claims	2	3
	Applicant Arguments/Remarks Made in an Amendment	4	7

Warnings:					
Information:					
2	Miscellaneous Incoming Letter	Bell_Declaration.pdf	3135451 72f899c4b9ebb134cbc750ee90c7d8018e7d2208	no	4
Warnings:					
Information:					
		Total Files Size (in bytes):	3285940		

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.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: January 24, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888 Confirmation No.: 2558

Filed: September 9, 2016 Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Office of Initial Patent Examination's Filing Receipt Corrections
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CORRECTED FILING RECEIPT

Dear Sir:

Applicant hereby requests that a corrected Filing Receipt be issued in the above-identified patent application to reflect an update to the Inventor information.

Please correct the Inventor(s) information from:

Leonard BELL, Woodbridge, CT;

Russell P. ROTHER, Oklahoma City, OK;

to:

Leonard BELL, Woodbridge, CT;

Russell P. ROTHER, Oklahoma City, OK;

Mark J. EVANS, Radnor, PA;

Applicant additionally requests that all pertinent U.S. Patent and Trademark Office records relating to the subject application be changed to reflect this correction.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3.

Dated: January 24, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicant

Electronic Patent Application Fee Transmittal

Application Number:	15260888
Filing Date:	09-Sep-2016
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
LATE FILING FEE FOR OATH OR DECLARATION	1051	1	140	140
PROCESSING FEE, EXCEPT PROV. APPLS.	1830	1	140	140
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**280**

Electronic Acknowledgement Receipt	
EFS ID:	28145796
Application Number:	15260888
International Application Number:	
Confirmation Number:	2558
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3
Receipt Date:	24-JAN-2017
Filing Date:	09-SEP-2016
Time Stamp:	15:17:03
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$280
RAM confirmation Number	012517INTEFSW00001795120080
Deposit Account	120080
Authorized User	Jill Sloper
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 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	Application Data Sheet	AXJ_114BUSCN3_Corrected_ADS.pdf	63084 c217b624f8d4835bf39fb9c864a7a02046d8a8d2	no	3
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
2	Oath or Declaration filed	Evans_Declaration.pdf	1266858 9ba0498a444335560b764fa8f9d6da0eddaa7951	no	1
Warnings:					
Information:					
3	Request under Rule 48 correcting inventorship	AXJ_114BUSCN3_Request_to_correct_inventorship.pdf	109059 660761a4c159701e19e72e9cfebab29c77c99b69	no	2
Warnings:					
Information:					
4	Request for Corrected Filing Receipt	AXJ_114BUSCN3_Request_for_corrected_FR.pdf	110739 d0779b728c4220cfaaac6da4bf42fb222aa85fd4	no	2
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	32085 66e1ad130ef8268741b2e2f052f71baddbd1c39b	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1581825		

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.

Corrected Application Data Sheet

Inventor Information

Inventor Number::	1
Given Name::	Leonard
Family Name::	BELL
City of Residence::	Woodbridge
State or Province of Residence::	CT
Country of Residence::	US
Street of mailing address::	59 Tumblebrook Road
City of mailing address::	Woodbridge
State or Province of mailing address::	CT
Country of mailing address::	US
Postal or Zip Code of mailing address::	06525

Inventor Number::	2
Given Name::	Russell
Middle Name::	P.
Family Name::	ROTHER
City of Residence::	Oklahoma City
State or Province of Residence::	OK
Country of Residence::	US
Street of mailing address::	10325 Forest Meadow Drive
City of mailing address::	Oklahoma City
State or Province of mailing address::	OK
Country of mailing address::	US

Postal or Zip Code of mailing address::	73151
Inventor Number::	3
Given Name::	Mark
Middle Name::	J.
Family Name::	EVANS
City of Residence::	Radnor
State or Province of Residence::	PA
Country of Residence::	US
Street of mailing address::	617 Twinbridge Drive
City of mailing address::	Radnor
State or Province of mailing address::	PA
Country of mailing address::	US
Postal or Zip Code of mailing address::	19087

Correspondence Information

Correspondence Customer Number::	133485
E-Mail address::	IPBoston.Docketing@nelsonmullins.com

Application Information

Application Number::	15/260,888
Filing Date::	09/09/16
Title::	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

PATIENTS BY AN INHIBITOR OF COMPLEMENT

Attorney Docket Number:: AXJ-114BUSCN3

Signature:

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

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

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

Signature	/Jill Gorny Sloper, Esq./	Date (YYYY-MM-DD)	2017-01-24
Name	Jill Gorny Sloper, Esq.	Registration Number	60,760

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

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

Title of Invention	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

As the below named inventor, I hereby declare that:

This declaration is directed to:

- [ ] The attached application, or
- [x] United States application or PCT international application number 15/260,888 filed on 09/09/2016.

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

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

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

WARNING:

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

LEGAL NAME OF INVENTOR

Inventor: Mark J. Evans Date (Optional): 22 Jan 2017

Signature: Mark J. Evans

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

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: January 24, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888 | Confirmation No.: 2558

Filed: September 9, 2016 | Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT | Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST TO CORRECT INVENTORSHIP UNDER 37 C.F.R. 1.48(a)

Dear Sir:

This request under 37 CFR § 1.48(a) is to add the following inventor to the above-referenced patent application:

Mark J. EVANS

This inventor was erroneously omitted from the above-referenced patent application at the time of filing.

Enclosed is a Corrected Application Data Sheet reflecting the names of all inventors in this application. A Request for Corrected Filing Receipt is also being submitted with this Request.

Submitted herewith is the processing fee of $140.00 pursuant to 37 CFR § 1.17(i)(1). Please charge any additional fees to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3, from which the undersigned is authorized to draw.

Dated: January 24, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicant

504193544 01/24/2017

PATENT ASSIGNMENT COVER SHEET

Electronic Version v1.1
Stylesheet Version v1.2

EPAS ID: PAT4240219

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

CONVEYING PARTY DATA

Name	Execution Date
MARK J. EVANS	01/23/2017

RECEIVING PARTY DATA

Name:	ALEXION PHARMACEUTICALS, INC.
Street Address:	100 COLLEGE ST.
City:	NEW HAVEN
State/Country:	CONNECTICUT
Postal Code:	06510

PROPERTY NUMBERS Total: 1

Property Type	Number
Application Number:	15260888

CORRESPONDENCE DATA

Fax Number: (617)217-4699

Correspondence will be sent to the e-mail address first; if that is unsuccessful, it will be sent using a fax number, if provided; if that is unsuccessful, it will be sent via US Mail.

Phone: (617) 217-4700
Email: IPBoston.Docketing@nelsonmullins.com, danielle.gamache@nelsonmullins.com
Correspondent Name: NELSON MULLINS RILEY & SCARBOROUGH LLP
Address Line 1: ONE POST OFFICE SQUARE
Address Line 4: BOSTON, MASSACHUSETTS 02109-2127

ATTORNEY DOCKET NUMBER:	AXJ-114BUSCN3
NAME OF SUBMITTER:	JILL GORNY SLOPER, ESQ.
SIGNATURE:	/Jill Gorny Sloper, Esq./
DATE SIGNED:	01/24/2017
	This document serves as an Oath/Declaration (37 CFR 1.63).

Total Attachments: 2
source=Evans_Assignment#page1.tif
source=Evans_Assignment#page2.tif

ASSIGNMENT BY INVENTOR

I, Mark J. EVANS, of Radnor, Pennsylvania, for good and valuable consideration paid to me by

Alexion Pharmaceuticals, Inc.

a Corporation of Delaware, having its principal place of business at 100 College St., New Haven, Connecticut 06510, the receipt of which is hereby acknowledged, do hereby sell, assign and transfer unto said

Alexion Pharmaceuticals, Inc.

its successors and assigns, the entire interest for the United States of America and all foreign countries including all rights of priority under the International Convention for the Protection of Industrial Property in a certain invention or improvement in

TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

described in an application

☐ Executed by me of even date herewith and about to be filed

or

☒ Application No. 15/260888, filed on September 9, 2016

in the United States Patent and Trademark Office, and in all Letters Patent of the United States and all foreign countries which may or shall be granted on said invention, or any parts thereof, or on said application, or any divisional, continuing, reissue or other applications based in whole or in part thereon. And I agree, for myself and my executors and administrators, with said Corporation and its successors and assigns but at its or their expense and charges, hereafter to execute all applications, amended specifications, deed or other instrument, and to do all acts necessary or proper to secure the grant of Letters Patent in the United States and in all other countries to said Corporation, with specifications and claims in such form as shall be approved by the counsel of said Corporation and to vest and confirm in said Corporation, its successors and assigns, the legal title to all such patents.

And I do hereby authorize and request the Commissioner of Patents and Trademarks of the United States to issue such Letters Patent as shall be granted upon said application or applications based thereon to said Corporation, its successors and assigns.

And I do further hereby grant the attorneys/agents of Nelson Mullins Riley & Scarborough LLP, the power to insert on this Assignment the filing date and/or application number of said application.

Mark J. Evans

Date: Jan 23, 2017

United States of America)
State of Pennsylvania) ss.:
County of Chester)

On this 23 day of January, 2017 before me, the the undersigned notary public, personally appeared Mark J Evans (name of document signer), proved to me through satisfactory evidence of identification, which was PA Drivers License, to be the person whose name is signed on the preceding or attached document in my presence.

Susanne B Martin (official signature and seal of notary)

My commission expires 10-04-2017

COMMONWEALTH OF PENNSYLVANIA
NOTARIAL SEAL
SUSANNE B. MARTIN, Notary Public
Easttown Twp., Chester County
My Commission Expires October 4, 2017

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 01/26/2017

MTEKLEMI SALE #00000011 Mailroom Dt: 01/24/2017 120080 15260888
01 FC : 1819 600.00 DA

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3

CONFIRMATION NO. 2558

133485
Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

37 CFR 1.48 ACKNOWLEDGEMENT LETTER

OC00000008747435

Date Mailed: 01/30/2017

NOTICE OF ACCEPTANCE OF REQUEST UNDER 37 CFR 1.48(a)

This is in response to the applicant's request under 37 CFR 1.48(a) submitted on 01/24/2017.

The request under 37 CFR 1.48(a) to correct the inventorship, to correct or update the name of an inventor, or to correct the order of names of joint inventors is accepted.

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

/ctuazon/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
15/260,888	09/09/2016	1644	1740	AXJ-114BUSCN3	10	1

CONFIRMATION NO. 2558

UPDATED FILING RECEIPT

133485
Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

OC000000088747434

Date Mailed: 01/30/2017

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Leonard BELL, Woodbridge, CT;
Russell P. ROTHER, Oklahoma City, OK;
Mark J. EVANS, Radnor, PA;

Applicant(s)

Alexion Pharmaceuticals, Inc., New Haven, CT;

Power of Attorney: The patent practitioners associated with Customer Number 133485

Domestic Priority data as claimed by applicant

This application is a CON of 15/148,839 05/06/2016
which is a CON of 13/426,973 03/22/2012 ABN
which is a CON of 12/225,040 05/13/2009 ABN
which is a 371 of PCT/US2007/006606 03/15/2007

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

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

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

If Required, Foreign Filing License Granted:

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/260,888**

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No

Title

TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Preliminary Class

424

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

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

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

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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Jan 30, 2017 03:40:55 AM

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Application	Document	Mailroom Date	Attorney Docket No.
15260888	R48.REQ.G	01/30/2017	AXJ-114BUSCN3
	APP.FILE.REC	01/30/2017	AXJ-114BUSCN3

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3	2558

133485 7590 02/02/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
02/02/2017	ELECTRONIC

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

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

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

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Applicant-Initiated Interview Summary	**Application No.** 15/260,888	**Applicant(s)** BELL ET AL.	
	Examiner JAMES ROGERS	**Art Unit** 1644	

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

(1) *JAMES ROGERS*.

(2) *DANIEL KOLKER, SPE*.

(3)*JILL SLOPER, JANE REMILLARD*.

(4)*Todd Spaldin*
(5) Darrell Fontenot
(6) Tara Rahemb
.

Date of Interview: *25 January 2017*.

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: *1*.

Identification of prior art discussed: _____.

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 outstanding 102(b) and 103(a) rejections of record were discussed. Applicant pointed out that the heavy chain sequence disclossed in the secondary reference Thomas is not the same as the currently claimed SEQ ID NO: 2 and pointed to the declaration of Leonard Bell, item (5) which provides a sequence alignment. No agreement was reached.

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

/JAMES ROGERS/ Examiner, Art Unit 1644	/DANIEL E. KOLKER/ Supervisory Patent Examiner, Art Unit 1644

Samsung Bioepis Exhibit No. 1002
Page 1113

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.

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Feb 02, 2017 03:46:51 AM

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Application	Document	Mailroom Date	Attorney Docket No.
15260888	INTV.SUM.APP	02/02/2017	AXJ-114BUSCN3

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133485 7590 04/11/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
04/11/2017	ELECTRONIC

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ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 15/260,888	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	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* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

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

Status

1)☒ Responsive to communication(s) filed on *1/19/2017*.

☐ 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) *1-10* 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) *1-10* 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/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

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

4) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-10 pending and under consideration in the instant examination.

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

In view of the Response filed 1/19/2017, the following rejections are maintained.

Response to Arguments

Claim Rejections - 35 USC § 102

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

> A person shall be entitled to a patent unless –
>
> (b) *the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.*

4. Claims 1-2 and 7-10 are rejected under 35 U.S.C. 102(b) as being anticipated by Hillmen et al. (N Engl J Med 2004; 350: 552-9, IDS Ref. of 10/21/2016), as is evidenced by applicant's specification as filed at p.44 and Thomas et al. (Molecular Immunology, 33(17/18), pp.1389-1401, 1996).

With respect to base claim 1, Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶).

While Hillmen does not specifically recite that the antibody that binds C5 comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO:4, the specification as filed at p. 44 discloses that SEQ ID NO: 2 and SEQ ID NO: 4 constitute the H and L chains for "eculizumab" and Hillmen, as noted supra, teaches administering "eculizumab". Hillmen further teaches that "eculizumab" is a recombinant humanized antibody that binds to C5 (p. 553, 3rd ¶) and cites Thomas which in turn describes in detail humanization of the clone referred to as "5G1.1" arrive at the humanized h5G1.1 which retain both the affinity and blocking functions of the murine 5g1.1 antibody (abstract). Accordingly and in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the L and H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Hillmen.

Claim 2 is included because Hillmen teaches that the patients received eculizumab by infusion (p. 554 under “Treatment Schedule").

Claim 7 is included because Hillmen teaches that the patients had previously been diagnosed of aplastic anemia (p. 555, 1st ¶).

Claim 8 is included because Hillmen teaches that patients received infusions of eculizumab (600 mg) every week for four weeks, followed one week later by a 900 mg dose and then by a dose of 900 mg every other week through week 12 (p. 552 under “methods” and p. 554 under “treatment schedule”).

Claims 9-10 are included because Hillmen teaches that a decrease in lactate dehydrogenase began after a single dose of eculizumab (immediate decrease occurs within 1 week of administration of the antibody) in all patients and remained within or just above the normal range for the duration of the study (sustained decrease in mean levels of LDH) (p. 555, last ¶).

The reference teachings anticipate the claimed invention.

Applicant’s arguments submitted on 1/19/2017 were fully considered but were not considered persuasive.

Applicant has submitted a declaration by Dr. Leonard Bell that prior to March 15, 2007 effective filing date of the present application, the complete structure of eculizumab was not disclosed in the prior art; nor was it available to the public and that Hillmen fails to teach any part of the sequence of eculizumab (p. 4 of the Response).

This argument was not considered persuasive for the following reasons:

The Bell declaration submitted on 1/19/2017 states that clinical trials were conducted “but only under strict control and conditions of confidentiality”. However the declaration does not provide any evidence that the experiments conducted in the HILLMEN reference were under any condition of confidentiality, nor does it provide any evidence that the authors of that study were working under the direction of the present inventors.

It remains the examiner’s position that there is substantial evidence that eculizumab was in use more than a year before applicant filed for the patent.

As noted supra, 35 USC 102(b) states:

A person shall be entitled to a patent unless —

(b) the invention was patented or described in a printed publication in this or a foreign country **or in public use or on sale in this country**, more than one year prior to the date of the application for patent in the United States,

While it is true that if the antibody was unknown prior, then a reference which names it does not enable that antibody. See MPEP 2121.02 sections I and II. See also 2121.01, "the stated test is whether a reference contains an 'enabling disclosure'... ." In re Hoeksema, 399 F.2d 269, 158 USPQ 596 (CCPA 1968). The disclosure in an asserted anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation." And … "Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his [or her] own knowledge to make the claimed invention." In re Donohue, 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985)."

In the instant case, applicant's position is that the mere naming is insufficient. However, the extensive number of both patent literature and NPL documents as well as clinical trials indicates that eculizumab was widespread and well-known. As evidence of the availability and use of eculizumab the examiner refers applicant to the following documents, some of which (exhibits A-E) are part of the Request for additional Information which is also part of this OA:

A) **Exhibit A:** from ClinicalTrails.gov (studies 41-60).
B) **Exhibit B:** Clinicaltrials.gov from 6/23/2005 for NCT00098280
C) **Exhibit C:** "Safety and Effectiveness of h5G1.1-mAb for dermatomyositis" clinical Trials.gov identifier NCT00005571
D) **Exhibit D:** Kaplan 2002. Current Opinion in Investigational Drugs 3(7):1017-1023.
E) **Exhibit E:** Appel et al. Kidney International 2006 70:S45-S50, in particular the paragraph spanning pp. S48-S49 (http://www.kidney-international.org/article/S0085-2538(15)51857-7/pdf)
F) **Wang (US 2005/0271660)**, which references pexelizumab or eculizumab as an antibody therapeutic (¶s4 , claim 7) and is presumed to be enabled.
G) **Rother (WO 2005/110481)**. See for example claims 10, 44-45, 79-80, 123, 125, 130 as well as page 17 line 26 – p. 18 line 12 (noting that the antibody from US Patent 6355245 is now named eculizumab).
H) **Rother (US 2009/0028850)**. This is the publication of the national-stage entry of the '481 application above. Note that the underlying application (11/596382) contains claims to methods of using eculizumab; the office has considered these claims to be enabled. Note that the case recently was decided by the PTAB, and the Board agreed that claims drawn to administering eculizumab were obvious (and thus presumably enabled and operative) as of the filing date of that underlying application, 5/16/2005.

In re Antor Media Corp (CAFC, 2012) states that subject matter disclosed in a reference, whether it is a patent claim, other parts of a patent, or non-patent literature, is presumed to be enabling absent evidence to the contrary.

It remains the examiner's position that eculizumab was effectively in the public domain over a year prior to the filing of this application, as indicated by the preponderance of evidence.

Claim Rejections - 35 USC § 103

5. 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 even 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 1-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 552-9), in view of Thomas et al. (Molecular Immunology, 33(17/18), pp.1389-1401, 1996) and of Evans et al. (US 6,355,245 B1, issued 3/12/2002), and further in view of Wang (US 2005/0271660).

The prior art teachings of Hillmen are discussed supra. The prior art teachings of Thomas and the '245 patent were discussed in the prior OA.

The prior art teachings differ from the claimed invention in the recitation that the antibody is administered at a dosage level of between 5-50 mg/kg per patient per treatment as in claim 3, that the composition is in a single unit dosage form as in claim 4 and that the single unit dosage form is a 300 mg single unit dosage form as in claim 5.

The prior art teachings further differ in the recitation that the pharmaceutical composition comprises a 300 mg single-use formulation of 30 ml of a 10 mg/ml sterile, preservative free solution as in claim 6.

The ‘245 patent further teaches antibody 5G1.1, which is the same as eculizumab (discussion supra), is effective at doses of 2.2, 3, or 5 mg per kg body weight; this corresponds to 154, 210, or 350 mg of antibody for a 70kg adult human (‘245 patent, column 16 lines 10-30). Evans (the ‘245 patent) also teaches that eculizumab is therapeutically effective when as little as 210 mg are present (column 16 lines 10-15). Evans teaches that the antibodies are particularly advantageous in that they are highly active even when administered in low doses (col 8 lines 25-32), providing a reasonable expectation of success in treating PHN with a dose less than what was disclosed by Hillmen. The reference teaches that it is within the skill of an artisan, particularly a physician, to determine the optimal dose (column 18 lines 15-30). Evans teaches formulations of the anti-C5 antibodies may be formulated in a pharmaceutically effective carrier such saline, which is considered to be a "preservative free solution". (col 18 lines 30-45).

Neither Hillmen nor the ‘245 patent (Evans) teaches a 300 mg single unit dosage as recited in the claims.

Nevertheless it would have been obvious to one of ordinary skill in the art to modify the teachings of Hillmen to administer 300 mg single unit dosages. Hillmen teaches that both 600 and 900 mg dosages can be used, and since Evans patent teaches that 210 or 350 mg of antibody would be expected to be therapeutically effective, arriving at the 300 mg dosage would be routine, convenient and obvious. One of ordinary skill in the art would have a reasonable expectation of success, as Evans teaches that the antibody exerts its effect at low doses.

It is further noted, [I]t is not inventive to discover the optimum or workable ranges by routine experimentation.” In re Aller, 220 F.2d 454, 456 (CCPA 1955); see also In re Peterson, 315 F.3d 1325 (Fed. Cir. 2003). “Only if the ‘results of optimizing a variable’ are ‘unexpectedly good’ can a patent be obtained for the claimed critical range.” In re Geisler, 116 F.3d 1465, 1469 (Fed. Cir. 1997) (quoting In re Antonie, 559 F.2d 618, 620 (CCPA 1977)). “[Discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art.” In re Boesch, 617 F.2d 272, 276 (CCPA 1980).

Finally, **Wang** teaches that eculizumab is highly soluble, including at up to 30 mg per ml (paragraph 0171, Figure 10). Wang further teaches that pharmaceutically acceptable carriers include pyrogen-free water may be used in formulation of the antibody (¶71) (sterile, preservative free solution). Therefore the artisan of ordinary skill would have had a reasonable expectation of success in making a 10 mg / ml solution of the antibody sterile, preservative free solution.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Applicant's arguments filed on 1/19/2017 were fully considered but were not considered persuasive.

Applicant's arguments mirror applicant's arguments with respect to the 35 USC 102(b) rejection over Hillmen supra. Accordingly, the examiner refers applicant to the examiner's rebuttal supra.

Request for Additional Information Under 37 CFR 1.105

7. Applicant and the assignee of this application are required under 37 CFR 1.105 to provide the following information that the examiner has determined is reasonably necessary to the examination of this application.

8. In response to this requirement, please provide answers to each of the following interrogatories eliciting factual information:

(A) **Studies #41-60 in the attached Exhibit A** provide prima facie evidence that the methods now claimed were in use in this country more than a year before the application for patent was filed. Therefore for each of studies #41-60 in the attached document Exhibit A, please answer the following questions:

(i) Whether any of the trials (#s41-60) used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at each of the study sites identified in (i) above for which the same sequences currently claimed was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers for those trials identified in (i) acting solely at the direction of the present inventors?

(B) **the study entitled "Safety and Effectiveness of h5G1.1-mAb for Dermatomyositis" listed on the attached Exhibit B** please answer the following questions:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at each the study sites was under any confidentiality agreement?

(ii) What was the nature of such confidentiality agreement in (ii) above?

(iii) Were the researchers acting solely at the direction of the present inventors?

(C) **Exhibit D: "Eculizumab Alexion –Mariana Kaplan**: please answer the following questions with respect to the study (445382) reference on p. 1018, 2nd ¶:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at the study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (i) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(D) **Exhibit E (Appel "new approaches to the treatment of glomerular diseases)**, please answer the following questions with respect to the randomized, placebo-controlled trial in 200 patients referenced at the end of p. S48:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(E) On what date was eculizumab as encompassed by the currently claimed invention (i.e., H chains SEQ ID NO: 2 and L chain SEQ ID NO: 4) first offered for sale?

9. The applicant is reminded that the reply to this requirement must be made with candor and good faith under 37 CFR 1.56. Where the applicant does not have or cannot readily obtain an item of required information, a statement that the item is unknown or cannot be readily obtained may be accepted as a complete reply to the requirement for that item.

Double Patenting

10. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *Jn re Berg,* 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *Jn re Goodman,* 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi,* 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum,* 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel,* 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321 (c) or 1.321 (d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(1)(1) - 706.02(1)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321 (b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

11. Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-19 of **US Patent No. 9,494,601,** view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Thomas et al. (Molecular Immunology, 33 (17/18), pp. 1389-1401), Evans et al. (US 6,355,245 81, issued 3/12/2002) and Wang (US 2005/0271660)**.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '601 claim eculizumab (see claim 10 of the '601).

The differences between the claimed invention and ‘601 (treatment of a patient suffering from PNH and recited doses) would be obvious in view of Hillmen, Thomas, Evan and Wang as discussed supra.

12. Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of **US Patent No. 9,409,980,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Thomas et al. (Molecular Immunology, 33 (17/18), pp. 1389-1401), Evans et al. (US 6,355,245 81, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '980 claim eculizumab (see claim 5 of the '980).

The differences between the claimed invention and ‘980 (treatment of a patient suffering from PNH and recited doses) would be obvious in view of Hillmen, Thomas, Evan and Wang as discussed supra.

13. Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-14 of **US Patent No. 9,556,263,** view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Thomas et al. (Molecular Immunology, 33 (17/18), pp. 1389-1401), Evans et al. (US 6,355,245 81, issued 3/12/2002) and Wang (US 2005/0271660)**.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '263 claim eculizumab (see claims 2 and 4 of the '263).

The differences between the claimed invention and ‘263 (treatment of a patient suffering from PNH and recited doses) would be obvious in view of Hillmen, Thomas, Evan and Wang as discussed supra.

14. No claim is allowed.

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you

have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

March 30, 2017 /JAMES ROGERS/
Art Unit 1644

/DANIEL E. KOLKER/

Supervisory Patent Examiner, Art Unit 1644

Notice of References Cited	Application/Control No. 15/260,888	Applicant(s)/Patent Under Reexamination BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	Page 1 of 3

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-2005/0271660 A1	12-2005	Wang, Yi	C07K16/18	424/144.1
*	B	US-2009/0028850 A1	01-2009	Rother; Russell P.	C07K16/18	424/133.1
	C	US-				
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N	WO 2005110481 A2	11-2005	CANADA	ZHONG ZHEN	C07K16/18
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Appel et al. "New approaches to the treatment of glomerular diseases" Kidney International 70, S45-S50 2006
	V	Hillmen et al. "Effect of Eculizumab on Hemolysis and Transfusion Requirements in Patients with Paroxysmal Nocturnal hemoglobinuria" NEJM, 350: 6, 2004
	W	69 Studies for eculizumab for clinicaltrials.gov, printed 2/28/2017, pp. 1-3
	X	Clinical Trials Identifier: NCT0098280, "Eculizumab to Treat Paroxysmal Nocturnal Hemoglobinuria" 6/23/2005, pp. 1-4

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

Notice of References Cited	Application/Control No. 15/260,888	Applicant(s)/Patent Under Reexamination BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	Page 2 of 3

U.S. PATENT DOCUMENTS

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FOREIGN PATENT DOCUMENTS

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	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Safety and Effectiveness of h5G1.1-mAb for dermatomyositis" clinicaltrials.gov identifier NCT00005571, April 25, 2000, pp. 1-4
	V	Alexion Mariana Kaplan "Eculizumab" Current Opinion in Investigational Drugs 2002 (3(7): 10107-1023
	W	
	X	

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

Notice of References Cited	Application/Control No. 15/260,888	Applicant(s)/Patent Under Reexamination BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	Page 3 of 3

U.S. PATENT DOCUMENTS

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	A	US-				
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FOREIGN PATENT DOCUMENTS

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Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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Trial record **1 of 1** for: nct00005571
Previous Study | Return to List | Next Study

Safety and Effectiveness of h5G1.1-mAb for Dermatomyositis

This study has been completed.

Sponsor:
National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

Information provided by:
National Institutes of Health Clinical Center (CC)

ClinicalTrials.gov Identifier:
NCT00005571

First received: April 25, 2000
Last updated: March 3, 2008
Last verified: December 2001
History of Changes

Full Text View | Tabular View | No Study Results Posted | Disclaimer | How to Read a Study Record

Purpose

This study will evaluate the safety and effectiveness of the experimental drug h5G1.1-mAb in treating patients with dermatomyositis. This disease, which causes skin rash, muscle weakness, and sometimes various other symptoms, may be due to an immune system abnormality. Drugs currently used to treat dermatomyositis, such as prednisone and various anticancer drugs, often have serious side effects and may not work in all patients. h5G1.1-mAb is a genetically engineered antibody that blocks the activity of certain proteins involved in the immune reaction that produces inflammation.

Patients age 18 years and older who have had dermatomyositis for at least 6 months and who have not improved with prednisone or other therapies, or who cannot tolerate prednisone or other therapies, may be eligible for this 12-week study. Candidates will have a history and physical examination, including blood and urine tests, throat culture, and muscle strength testing. Participants will be randomly assigned to receive either h5G1.1-mAb or placebo (an inactive substance). The drug or placebo will be given intravenously (through a thin tube inserted into a vein) once a week for five doses and then every other week for two more doses.

Participants will undergo the following additional tests at various intervals during the study as follows:

1. Complete physical examination (visit 9)
2. Blood and urine tests (various intervals)
3. Muscle strength testing, assessment of ability to perform daily tasks, and completion of questionnaire regarding functional abilities (visits 2, 6 and 9)
4. Ultrasound imaging of muscle (during certain muscle exercises) (visits 2, 6 and 9)
5. Electrocardiogram (EKG) (visits 2 and 9)
6. Throat swab (culture) (visit 6)
7. Examination and photography of skin lesions (visits 2 and 9)
8. Skin biopsy - removal of small sample of skin tissue under local anesthetic (visits 2 and 9)
9. Magnetic resonance imaging (MRI) scan of muscles (visits 2 and 9)
10. Possible muscle biopsy - removal of small sample of muscle tissue under local anesthetic (visits 2 and 9).

Condition	Intervention	Phase
Dermatomyositis	Drug: h5G1.1-mAb	Phase 2

Study Type: Interventional
Study Design: Primary Purpose: Treatment

Official Title: A Randomized, Third-Party-Blind, Placebo-Controlled Pilot Study of the Effect of h5G1.1-mAb on Dermatomyositis Patients

Resource links provided by NLM:

Drug information available for: Eculizumab

Genetic and Rare Diseases Information Center resources: Idiopathic Inflammatory Myopathy Dermatomyositis Polymyositis

U.S. FDA Resources

Further study details as provided by National Institutes of Health Clinical Center (CC):

Estimated Enrollment:	17
Study Start Date:	April 2000
Estimated Study Completion Date:	December 2001

Detailed Description:

This is a randomized, third-party, placebo-controlled pilot study of h5G1.1-mAb, an antibody directed at the complement component C5, administered to patients with dermatomyositis with persistent disease. We are one of the four groups taking part in a multicenter trial under the sponsorship of Alexion Pharmaceuticals, Inc.

In dermatomytosis, humorally-mediated damage to muscle and skin microvasculature appears very important. The deposition of the membrane attack complex (C5-9) on the capillaries has been shown to precede the destruction of muscle fibers and to be a specific finding in the skin lesion. These observations, along with the recent discovery of the effectiveness of intravenous gammaglobulin (IVIG) in dermatomyositis, support the role of complement activation in the pathogenesis of dermatomyositis. Among the activated components of the complement system, the products that are generated after cleavage of C5, namely, C5a and C5b-9, are potent inflammatory mediators with pleiotropic activities. Inhibition of complement activation at C5 would prevent the formation of these pro-inflammatory molecules while allowing the generation of C3b, which is critical for opsonization and immune complex clearance. C5 inhibition therefore represents a potentially effective therapeutic modality for dermatomyositis.

Anti-C5 monoclonal antibodies are designed to prevent the cleavage of C5. A murine monoclonal antibody to human C5, m5G1.1-mAb, was humanized (h5G1.1-mAb) by grafting the antibody's antigen-binding CDR regions onto human antibody-derived framework and constant domains. This antibody is being made available by Alexion Pharmaceuticals, and will be used under their IND.

There will be 15 patients entered (with the goal of 12 evaluable patients) distributed among two treatment groups. Patients will be randomized at a 3 to 1 ratio between h5G1.1-mAb and placebo. Following a screening period of no less than 7 days, a 2 month double-blind, randomized treatment period will commence. Patients will receive an active (h5G1.1-mAb, 8mg/kg) bolus infusion every week X 5, then every 2 weeks X 2, or a placebo bolus infusion every week X 5, then every 2 weeks X 2. All patients will be receiving 7 infusions of h5G1.1-mAb or placebo.

The objective is to determine the safety and tolerability of h5G1.1-mAb and to evaluate the pharmacokinetics and pharmacodynamics of this antibody as well as its efficacy in the target population. Safety will be assessed via the collection of adverse events and the evaluation of pre- and post-treatment laboratory data. Efficacy will be assessed by the change in the manual muscle testing score relative to placebo at 2.25 months (one week following the end of the active treatment period) (primary efficacy value), and the change in the muscle enzyme level, MRI findings, skin examination findings and biopsy, and SF-36 (secondary efficacy values).

Eligibility

Ages Eligible for Study:	Child, Adult, Senior
Sexes Eligible for Study:	All
Accepts Healthy Volunteers:	No

Criteria

Patients age greater than or equal to 18 years.

Patients with a diagnosis of definite or probable dermatomyositis by criteria of Bohan et. al., with either active rash typical of dermatomyositis, history of rash typical of dermatomyositis, or Gottron's papules.

Patients with a manual muscle testing score less than or equal to 136/170.

Patients with a disease duration greater than or equal to 6 months.

Patients with persistent disease (defined as active rash plus CK greater than or equal to 2 times ULN), or rapidly progressive disease, or response to steroids with inability to taper dose, or unacceptable side effects of steroids.

Patients may be on stable (times 28 days prior to Visit 2) dose of MTX or AZA.

No other immunosuppressive agents times 84 days prior to first dose.

Patients on stable oral steroid use for 28 days prior to Visit 2.

Patients with adequate hematologic function, defined as hemoglobin greater than or equal to 8.5 g/dl, WBC greater than or equal to 3,000 mm(3), neutrophils greater than or equal to 1,200 mm(3), platelets greater than or equal to 100,000 mm(3).

Patients must be willing and able to give informed consent.

Women must be post-menopausal, surgically sterile or practicing a medically approved method of contraception.

Patients with liver disease will be excluded.

Patients with history of alcohol or drug abuse within two years of screening, or a history of positive Hepatitis B or Hepatitis C serology, unless vaccinated will be excluded.

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Patients with renal insufficiency, defined as creatinine greater than or equal to 2.0 mg/dl will be excluded.

Patients with history of malignancy, except basal cell carcinoma and remote (greater than or equal to 5 years) malignancies in complete remission will be excluded.

Patients with history of poorly controlled diabetes will be excluded.

Patients with presence or suspicion of active infection, recent serious infection, or chronic/recurrent viral or bacterial infection will be excluded.

Patients with throat culture positive for pathogenic Neisseria species (meningitidis or gonorrhoeae) will be exluded. Subjects with a positive culture may be treated with appropriate antibiotic therapy and retested.

Patients with joint disease or replacement that would interfere with patient's ability to perform muscle testing will be excluded.

Patients with any clinically significant medical condition that is likely to interfere with the participation in the study or the evaluation of the study medication's safety profile will be excluded.

No patients with known or suspected hereditary complement deficiency will be excluded.

Patients with history of allergic reaction to murine proteins will be excluded.

Patients participating in any other investigational drug trial, or exposure to other investigational agent or device within thirty days prior to screening will be excluded.

Pregnant or breastfeeding patients will be excluded.

Women intending to conceive during the course of the study, including follow-up period will be excluded.

Patients with history of HIV, Lyme disease, or other environmentally induced myositis will be excluded.

Contacts and Locations

Choosing to participate in a study is an important personal decision. Talk with your doctor and family members or friends about deciding to join a study. To learn more about this study, you or your doctor may contact the study research staff using the Contacts provided below. For general information, see Learn About Clinical Studies.

Please refer to this study by its ClinicalTrials.gov identifier: NCT00005571

Locations

United States, Maryland

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
Bethesda, Maryland, United States, 20892

Sponsors and Collaborators

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

More Information

Publications:

Pearson CM, Bohan A. The spectrum of polymyositis and dermatomyositis. Med Clin North Am. 1977 Mar;61(2):439-57. Review.

Love LA, Leff RL, Fraser DD, Targoff IN, Dalakas M, Plotz PH, Miller FW. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. Medicine (Baltimore). 1991 Nov;70(6):360-74.

Adams EM, Pucino F, Yarboro C, Hicks JE, Thornton B, McGarvey C, Sonies BC, Bartlett ML, Villalba ML, Fleisher T, Plotz PH. A pilot study: use of fludarabine for refractory dermatomyositis and polymyositis, and examination of endpoint measures. J Rheumatol. 1999 Feb;26(2):352-60.

ClinicalTrials.gov Identifier:	NCT00005571 History of Changes
Other Study ID Numbers:	000117 00-AR-0117
Study First Received:	April 25, 2000
Last Updated:	March 3, 2008

Keywords provided by National Institutes of Health Clinical Center (CC):

Skin	Complement
Humoral Immunity	Antibody
Myositis	Dermatomyositis

Additional relevant MeSH terms:

Dermatomyositis	Neuromuscular Diseases
Polymyositis	Nervous System Diseases
Myositis	

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Muscular Diseases
Musculoskeletal Diseases
Connective Tissue Diseases
Skin Diseases

ClinicalTrials.gov processed this record on February 24, 2017

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Rank	Status	Study
41	Terminated	Safety & Efficacy of Eculizumab to Prevent AMR in Living Donor Kidney Transplant Recipients Requiring Desensitization **Condition:** Antibody Mediated Rejection **Intervention:** Drug: Eculizumab **Study Start:** September 2011 **First Received:** July 19, 2011
42	Withdrawn	Targeting Complement Activation in Antineutrophil Cytoplasmic Autoantibodies (ANCA)-Vasculitis - Eculizumab **Condition:** Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis **Interventions:** Drug: Standard of care treatment; Drug: eculizumab **Study Start:** May 2011 **First Received:** December 13, 2010
43	Completed	Safety and Efficacy Study of Eculizumab in Shiga-Toxin Producing Escherichia Coli Hemolytic-Uremic Syndrome (STEC-HUS) **Condition:** Shiga-like Toxin-producing Escherichia Coli **Intervention:** Drug: Eculizumab (Soliris®) **Study Start:** July 2011 **First Received:** August 3, 2011
44	Completed	Extension Study of Eculizumab in Patients With Transfusion Dependent Paroxysmal Nocturnal Hemoglobinuria (PNH) **Condition:** Paroxysmal Hemoglobinuria, Nocturnal **Intervention:** Drug: eculizumab **Study Start:** May 2005 **First Received:** July 20, 2005
45	Recruiting	Complement Inhibition Using Eculizumab to Overcome Platelet Transfusion Refractoriness in Patients With Severe Thrombocytopenia **Conditions:** Thrombocytopenia; Alloimmune Platelet Refractoriness **Intervention:** Drug: Eculizumab **Study Start:** November 4, 2014 **First Received:** November 21, 2014
46	Recruiting	Eculizumab in Shiga-toxin Related Hemolytic and Uremic Syndrome Pediatric Patients - ECULISHU **Condition:** Hemolytic Uremic Syndrome of Childhood **Interventions:** Drug: Eculizumab; Drug: Placebo **Study Start:** June 2015 **First Received:** July 28, 2014
47	Completed	Eculizumab to Treat Paroxysmal Nocturnal Hemoglobinuria

Condition: Hemoglobinuria, Paroxysmal
Intervention: Drug: Eculizumab
Study Start: December 2004
First Received: December 3, 2004

48 Completed — Eculizumab to Treat Paroxysmal Nocturnal Hemoglobinuria
Condition: Paroxysmal Hemoglobinuria, Nocturnal
Intervention: Drug: Eculizumab
Study Start: August 9, 2005
First Received: August 11, 2005

49 Completed — Study Using Eculizumab in Transfusion Dependent Paroxysmal Nocturnal Hemoglobinuria (PNH) Patients
Condition: Hemoglobinuria, Paroxysmal
Intervention: Drug: eculizumab
Study Start: October 2004
First Received: July 18, 2005

50 Completed — Study of Safety in Hemolytic Paroxysmal Nocturnal Hemoglobinuria (PNH) Patients Treated With Eculizumab
Condition: Hemoglobinuria, Paroxysmal
Intervention: Drug: eculizumab
Study Start: December 2004
First Received: July 19, 2005

51 Completed — Eculizumab in Treating Patients With Paroxysmal Nocturnal Hemoglobinuria
Condition: Leukemia
Intervention: Biological: eculizumab
Study Start: November 2004
First Received: June 2, 2005

52 Completed — Safety and Efficacy Study of Eculizumab in Patients With Mild Allergic Asthma
Condition: Allergic Asthma
Intervention: Drug: eculizumab
Study Start: September 2007
First Received: June 12, 2007

53 Completed — A Retrospective, Observational, Non-interventional Trial to Assess Eculizumab Treatment Effect in Patients With Atypical Hemolytic Uremic Syndrome (aHUS)
Condition: Atypical Hemolytic Uremic Syndrome (aHUS)
Intervention:
Study Start: August 2009
First Received: November 13, 2012

54 Unknown † — The Safety and Efficacy of Eculizumab in Japanese Patients With Atypical Hemolytic Uremic Syndrome (aHUS)
Condition: Atypical Hemolytic Uremic Syndrome (aHUS)
Intervention: Drug: Eculizumab
Study Start: May 2012
First Received: December 4, 2012

55 Completed — Safety and Effectiveness of h5G1.1-mAb for Dermatomyositis
Condition: Dermatomyositis
Intervention: Drug: h5G1.1-mAb
Study Start: April 2000
First Received: April 25, 2000

56 Completed — To Characterize the Safety and Tolerability of Eculizumab in Two Japanese aHUS Patients
Condition: ATYPICAL HEMOLYTIC UREMIC SYNDROME (AHUS)
Intervention:
Study Start: May 2012
First Received: December 4, 2012

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57	Not yet recruiting	Phase 2 Safety and Efficacy Study of RA101495 to Treat PNH Patients Who Have an Inadequate Response to Eculizumab **Condition:** Paroxysmal Nocturnal Hemoglobinuria (PNH) **Intervention:** Drug: RA101495 **Study Start:** February 2017 **First Received:** January 20, 2017
58	Recruiting	Identification and Treatment of Thrombotic Microangiopathies in Allogeneic Stem Cell Transplants **Conditions:** Thrombotic Microangiopathy; Disorder Related to Bone Marrow Transplantation **Intervention:** Drug: eculizumab **Study Start:** September 2014 **First Received:** October 15, 2015
59	Active, not recruiting	Paroxysmal Nocturnal Hemoglobinuria (PNH) Registry **Condition:** Paroxysmal Nocturnal Hemoglobinuria **Intervention:** **Study Start:** January 2007 **First Received:** April 15, 2011
60	Terminated	aHUS Observational Long Term Follow-Up **Condition:** Atypical Hemolytic Uremic Syndrome **Intervention:** **Study Start:** March 2012 **First Received:** January 18, 2012

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⏮ ◀ Previous Page (21-40) Studies Shown (41-60) Next Page (61-69) ▶ ⏭

† Study has passed its completion date and status has not been verified in more than two years.

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ORIGINAL ARTICLE

Effect of Eculizumab on Hemolysis and Transfusion Requirements in Patients with Paroxysmal Nocturnal Hemoglobinuria

Peter Hillmen, M.B., Ph.D., Claire Hall, M.B., Ch.B., Judith C.W. Marsh, M.B., M.D., Modupe Elebute, M.B., M.D., Michael P. Bombara, B.S., Beth E. Petro, B.S., Matthew J. Cullen, B.Sc., Stephen J. Richards, Ph.D., Scott A. Rollins, Ph.D., Christopher F. Mojcik, M.D., Ph.D., and Russell P. Rother, Ph.D.

ABSTRACT

BACKGROUND

Paroxysmal nocturnal hemoglobinuria (PNH) arises from a somatic mutation of the *PIG-A* gene in a hematopoietic stem cell and the subsequent production of blood cells with a deficiency of surface proteins that protect the cells against attack by the complement system. We tested the clinical efficacy of eculizumab, a humanized antibody that inhibits the activation of terminal complement components, in patients with PNH.

METHODS

Eleven transfusion-dependent patients with PNH received infusions of eculizumab (600 mg) every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12. Clinical and biochemical indicators of hemolysis were measured throughout the trial.

RESULTS

Mean lactate dehydrogenase levels decreased from 3111 IU per liter before treatment to 594 IU per liter during treatment (P=0.002). The mean percentage of PNH type III erythrocytes increased from 36.7 percent of the total erythrocyte population to 59.2 percent (P=0.005). The mean and median transfusion rates decreased from 2.1 and 1.8 units per patient per month to 0.6 and 0.0 units per patient per month, respectively (P=0.003 for the comparison of the median rates). Episodes of hemoglobinuria were reduced by 96 percent (P<0.001), and measurements of the quality of life improved significantly.

CONCLUSIONS

Eculizumab is safe and well tolerated in patients with PNH. This antibody against terminal complement protein C5 reduces intravascular hemolysis, hemoglobinuria, and the need for transfusion, with an associated improvement in the quality of life in patients with PNH.

From the Department of Haematology, Leeds Teaching Hospitals National Health Science Trust, Leeds, United Kingdom (P.H., C.H., M.J.C., S.J.R.); the Department of Haematology, St. George's Hospital Medical School, London (J.C.W.M., M.E.); and Alexion Pharmaceuticals, Cheshire, Conn. (M.P.B., B.E.P., S.A.R., C.F.M., R.P.R.). Address reprint requests to Dr. Hillmen at the Department of Haematology, Leeds General Infirmary, Great George St., Leeds LS1 3EX, United Kingdom, or at peter.hillmen@panp-tr.northy.nhs.uk.

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THE MAJOR CLINICAL SIGNS OF PAROXysmal nocturnal hemoglobinuria (PNH) are intravascular hemolysis, venous thrombosis, and hemoglobinuria.[1] The disease arises from a somatic mutation of the *PIG-A* gene in a pluripotent hematopoietic stem cell. *PIG-A* encodes a protein that is essential for the synthesis of glycosylphosphatidylinositol (GPI), a lipid moiety that is embedded in the plasma membrane, where it serves to anchor a wide variety of proteins to the cell surface. The mutant stem cell subsequently expands to form a hematopoietic clone with a deficiency in proteins that are normally attached to the cell surface by the GPI anchor.[2,3] The mature blood cells derived from the hematopoietic clone can have a complete deficiency (type III) or a partial deficiency (type II) of GPI-linked proteins and almost always coexist with residual cells with a normal expression of these proteins (previously identified as type I).

The clinical features of PNH result from the lack of one or more GPI-linked proteins that protect cells from complement-mediated attack. Two such proteins — CD55 and CD59 — are absent from PNH type III erythrocytes, platelets, and other blood cells.[4-6] CD55 regulates early complement activation by inhibiting C3 convertases,[7] whereas CD59 inhibits the assembly of the membrane-attack complex C5b–C9 by interacting with C8 and C9.[4,5] The lack of CD59 is probably responsible for the increased sensitivity of PNH erythrocytes and platelets to complement.[4,8-13]

Eculizumab is a recombinant humanized monoclonal antibody that was designed to block the activation of terminal complement components.[14,15] It binds specifically to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b, thereby preventing the release of the inflammatory mediator C5a and the formation of the cytolytic-pore C5b–C9. Blockade of the complement cascade at C5 preserves the early components of complement that are essential for the opsonization of microorganisms and clearance of immune complexes.[16] In this trial, we investigated whether eculizumab could reduce the incidence of intravascular hemolysis, hemoglobinuria, and transfusion requirements in patients with PNH.

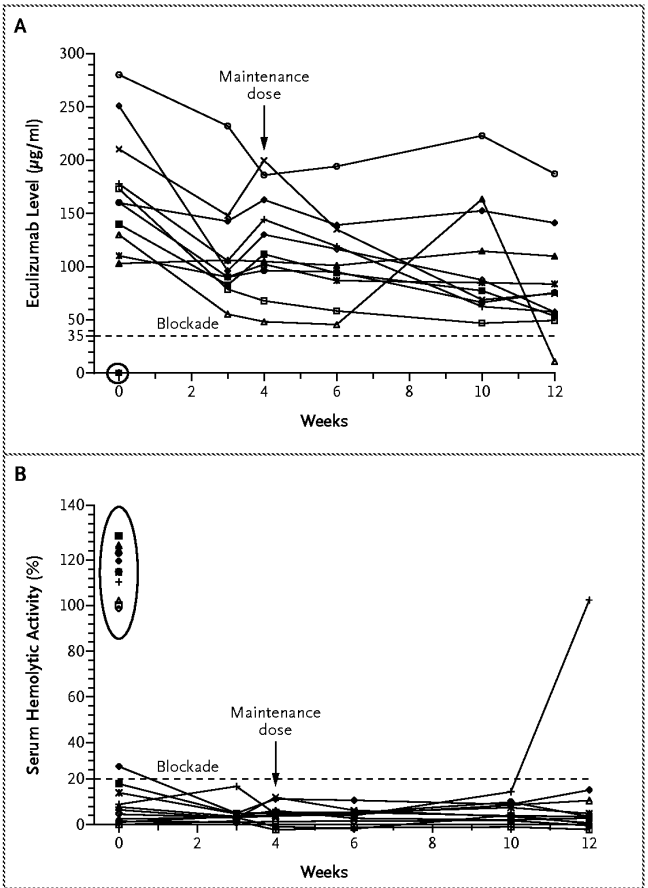

Figure 1. Pharmacokinetic and Pharmacodynamic Analysis of Eculizumab in 11 Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH).

Panel A depicts serum levels of eculizumab during the 12-week treatment period. Eculizumab levels just before a patient's first dose of eculizumab are circled on the x axis. Also shown at time zero are levels of eculizumab one hour after the initial dose. Other data points indicate trough levels of eculizumab for each patient at weeks 3, 4, 6, 10, and 12. The beginning of the 900-mg maintenance dose is indicated by an arrow. The level of eculizumab required to block complement activity (≥35 μg per milliliter) is indicated by a dashed line. Panel B shows hemolytic activity in serum during the 12-week treatment period, as assessed in a presensitized chicken-erythrocyte hemolytic assay. Pretreatment serum hemolytic activity values are circled at time zero. Also shown at time zero are values for hemolytic activity one hour after the initial dose. Other data points indicate trough hemolytic activity at weeks 3, 4, 6, 10, and 12. The dashed line indicates the level of hemolytic activity that is considered to represent complete complement blockade (≤20 percent).

METHODS

PATIENTS

The study was conducted from May through December 2002. Men and women (18 years of age and older) who had received a diagnosis of PNH at least six months earlier, had a detectable GPI-deficient hematopoietic clone, and had received at least four red-cell transfusions in the preceding 12 months were

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eligible. Patients were required to have a negative throat culture for *Neisseria meningitidis* and *N. gonorrhoeae*. All patients were vaccinated against *N. meningitidis* (Mengivac (A+C), Aventis Pasteur) before treatment. One patient had a stroke after consent but never received eculizumab and was excluded. Patients who were taking stable doses of immunosuppressive drugs (e.g., cyclosporine), warfarin, and iron supplements were permitted to continue them.

The trial was approved by the local research ethics committee and was performed according to the International Conference on Harmonisation and Good Clinical Practice Standards. Eleven patients gave written informed consent and were treated with eculizumab.

TREATMENT SCHEDULE

Patients received infusions of 600 mg of eculizumab weekly for four weeks, followed one week later by a 900-mg dose and then by a dose of 900 mg every other week through week 12.

INVESTIGATIONS

In this open-label pilot study, we obtained data on the pharmacokinetics, pharmacodynamics, and immunogenicity of eculizumab and observed its clinical effects by measuring the following: lactate dehydrogenase, haptoglobin, bilirubin, and hemoglobin levels; reticulocyte counts; the proportion of GPI-deficient cells, as assessed by flow cytometry[17]; the rate of transfusion with packed red cells; the rate of occurrence of hemoglobinuria (assessed by daily comparison of the first morning urine sample with a standardized color chart before and during treatment); and the quality of life, as reflected by the scores on the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 instrument. The trigger for transfusion during the study period remained unchanged for each patient, as compared with their care before entry into the study: patients received blood transfusions when they had symptoms resulting from anemia.

Assessment of the safety of eculizumab included ascertainment of treatment-related adverse events, electrocardiography, and routine laboratory tests (e.g., serum chemical analyses and complete blood counts).

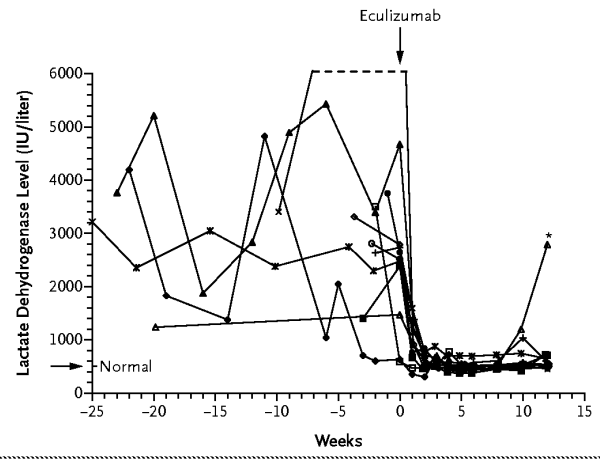

Figure 2. Analysis of Lactate Dehydrogenase Levels, a Biochemical Indicator of Hemolysis, in 11 Patients with Paroxysmal Nocturnal Hemoglobinuria up to 25 Weeks before and during 12 Weeks of Eculizumab Treatment.

The first dose of eculizumab is indicated by an arrow, as is the upper limit of the normal range of lactate dehydrogenase at the Leeds Teaching Hospital. The data point identified at week 12 by the asterisk represents a reading that was obtained from a duplicate serum sample since the original sample was lost. The dashed line represents off-scale points from one patient with a peak value of 12,100 IU per liter.

ASSAY METHODS

The pharmacokinetics of eculizumab were determined with an enzyme-linked immunosorbent assay that detects both free and C5-bound eculizumab.[15] The pharmacodynamics of eculizumab were determined by measuring the capacity of the patient's serum to lyse chicken erythrocytes in a standard total human serum-complement hemolytic assay.[18] The presence or absence of antibodies against eculizumab was assessed by an enzyme-linked immunosorbent assay.[19]

STATISTICAL ANALYSIS

Biochemical values were compared with the use of a paired Student's t-test, quality-of-life measurements with the use of a mixed-effect analysis of covariance, the median rate of transfusions with the use of a Wilcoxon signed-rank test, and the comparison of the number of days with paroxysms with the use of Fisher's exact test.

The corresponding author and the sponsor were jointly responsible for the design of this trial and the development of the protocol. Data were collected and analyzed by a clinical research organization, Kendle International, which maintained the trial data base and provided statistical support. The manuscript was prepared by the corresponding author, with substantial review and comments by the other authors and the sponsor. Final decisions on

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the content of the manuscript rested with the corresponding author in consultation with the other authors. All authors had access to the primary data.

RESULTS

DEMOGRAPHIC CHARACTERISTICS

Six men and five women (median age, 48 years; range, 21 to 67) with a median duration of PNH of 8.6 years (range, 1.7 to 37.4) participated in the trial. Five of the patients had platelet counts at base line of less than 150,000 per cubic millimeter. Eight patients had previously received a diagnosis of aplastic anemia, two were concomitantly receiving cyclosporine for aplastic anemia, and six were receiving warfarin.

SAFETY

All patients completed the 12-week study. There were no deaths or thrombotic events, and all patients subsequently entered a 12-month extension study. Each patient reported one or more adverse events during the trial. Events reported by three patients included headache and upper respiratory tract infection. Events reported by two patients included influenza-like symptoms, rigors, dizziness, nausea, nasal congestion, and joint aches. None of these events were attributed to the study medication. Serious adverse events occurred in two patients. The first was hospitalized with a viral chest infection. The second reported nausea, vomiting, and headache after the first infusion, with dizziness and shivering the following day. The patient was hospitalized overnight, and subsequent infusions were well tolerated.

PHARMACOKINETICS, PHARMACODYNAMICS, AND IMMUNOGENICITY OF ECULIZUMAB

Peak and trough levels of eculizumab were well above 35 μg per milliliter from one hour after the first dose through the completion of the 600-mg weekly dose period (Fig. 1A). In 10 patients, serum trough levels of eculizumab remained above 35 μg per milliliter for the entire study.

The hemolytic activity of serum from these 10 patients was completely blocked (less than 20 percent in the chicken–red-cell assay) for essentially the entire treatment period (Fig. 1B). In 1 of the 11 patients, the trough level of eculizumab fell below 35 μg per milliliter at week 12 and serum hemolytic activity returned. In no case were antibodies against eculizumab detected.

BIOCHEMICAL INDICATORS OF HEMOLYSIS

Levels of lactate dehydrogenase in serum were markedly elevated in all patients before eculizumab treatment. Mean (±SD) lactate dehydrogenase levels fell from 3111±598 IU per liter during the 12 months before enrollment to 594±32 IU per liter (normal range, 150 to 480) during treatment ($P=0.002$) (Fig. 2).

The decrease in lactate dehydrogenase began after a single dose of eculizumab in all patients. Lactate dehydrogenase levels remained within or just above the normal range for the duration of the study (Fig. 2). In the one patient in whom eculizumab levels fell below 35 μg per milliliter at week 12 (Fig. 1A), hemolytic activity returned (Fig. 1B) and lactate dehydrogenase levels increased transiently (Fig. 2).

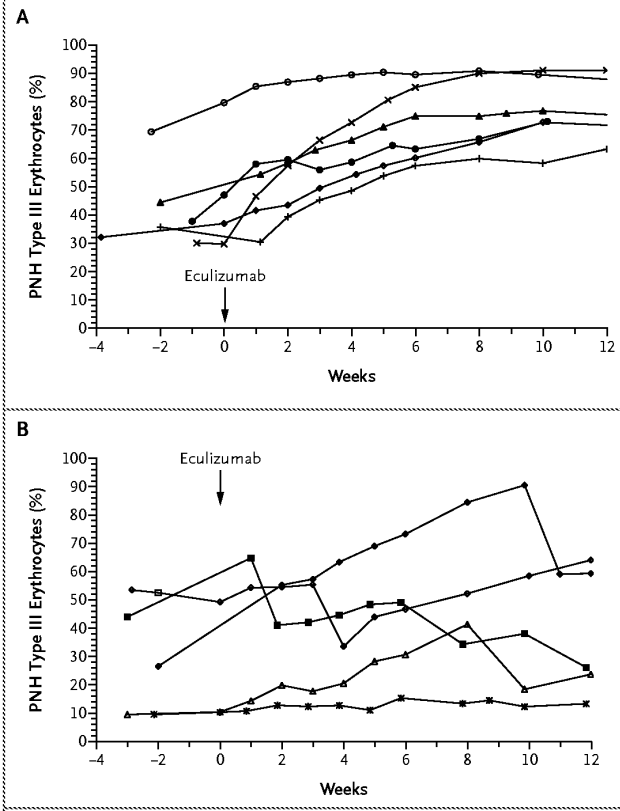

Figure 3. Changes in the Percentage of Paroxysmal Nocturnal Hemoglobinuria (PNH) Type III Erythrocytes during Eculizumab Treatment in Six Patients Who Received No Transfusions during Treatment (Panel A) and Five Patients Who Received at Least One Transfusion after Starting Treatment (Panel B).
The initial dose of eculizumab is indicated by the arrow in each panel.

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The dosing frequency was increased from 900 mg every 14 days to 900 mg every 12 days, reestablishing complete complement blockade during the ongoing 12-month extension study (data not shown).

Haptoglobin became detectable in the serum of 5 of the 11 patients after two weeks of eculizumab treatment but returned to undetectable levels soon thereafter (data not shown). Bilirubin levels were also elevated in most patients at base line and did not change significantly during treatment (data not shown).

EFFECT ON PNH CLONES

Type III erythrocytes are highly sensitive to lysis by complement and as a result have a short life span. In our study, the percentage of type III erythrocytes increased significantly from a mean of 36.7±5.9 percent before treatment to 59.2±8.0 percent at the end of 12 weeks of treatment (P=0.005) (Fig. 3). The increase was particularly consistent in six patients who remained transfusion-independent during the study (Fig. 3A). In patients who received transfusions during the study, sudden drops in the proportion of type III cells were seen as the transfused red cells diluted the type III population (Fig. 3B). There were no significant changes in the percentages of PNH type III neutrophils, monocytes, or platelets during treatment with eculizumab; in most cases, these percentages were 90 to 100 percent before the study (data not shown).

TRANSFUSION REQUIREMENTS, HEMOGLOBIN LEVELS, AND RETICULOCYTE COUNTS

During the year preceding enrollment, the range of red-cell transfusions received by the 11 patients was 12 to 55 units, whereas during the three months of the study, the range was 0 to 8 units. Before eculizumab treatment, the mean and median transfusion rates were 2.1 and 1.8 units per patient per month, respectively (Table 1). These transfusion rates decreased to 0.6 and 0.0 unit per patient per month, respectively, during the three months of treatment with eculizumab (P=0.003). Hemoglobin levels did not increase significantly during the treatment period, although hemoglobin values in six patients stabilized without transfusions (Table 1). Similarly, the numbers of reticulocytes remained relatively constant during eculizumab treatment.

HEMOGLOBINURIA

Our patients recorded the color of their urine each morning using a color chart designed to assess the

Table 1. **Transfusion Rates, Hemoglobin Levels, and Reticulocyte Counts 12 Months before and after 3 Months of Eculizumab Treatment.**

Patient No.	12 Mo before Eculizumab Treatment				After 3 Mo of Eculizumab Treatment			
	Transfusions		Hemoglobin	Reticulocytes	Transfusions		Hemoglobin	Reticulocytes
	no. of units	*rate**	*g/dl*	*×10⁻³/mm³*	*no. of units*	*rate†*	*g/dl*	*×10⁻³/mm³*
1	22	1.8	10.3	77.5	2	0.7	10.0	100.7
2	23	1.9	8.3	200.0	8	2.9	8.8	182.6
3	20	1.6	10.1	169.5	0	0.0	10.7	175.9
4	28	2.3	9.3	282.0	0	0.0	9.4	333.3
5	12	1.0	11.9	96.3	2	0.7	10.6	121.8
6	14	1.2	9.8	346.8	0	0.0	10.6	259.0
7	34	2.8	12.8	100.6	0	0.0	13.5	166.8
8	21	1.7	9.5	164.5	0	0.0	9.8	239.6
9	55	4.5	10.7	138.0	3	1.1	11.4	285.8
10	41	3.4	8.5	108.7	5	1.8	8.8	140.1
11	14	1.2	8.5	91.4	0	0.0	10.0	97.4
Median‡		1.8				0.0		
Mean		2.1	10.0	161.4		0.6	10.3	191.2

* The rate (in units per month) was calculated as (number of units÷365 days)×30.
† The rate (in units per month) was calculated as (number of units÷84 days)×30.
‡ P=0.003 for the change in the median rate of transfusion by the Wilcoxon signed-rank test.

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degree of hemoglobinuria (Fig. 4A) during both the 2-to-4-week screening period and the 12-week treatment period. Paroxysms of hemoglobinuria were prospectively defined as dark-colored urine with a colorimetric level of 6 or more. In nine patients for whom urine scores were assessed, the mean incidence of paroxysms was reduced from 2.9 days to 0.12 day per patient per month ($P<0.001$) (Fig. 4B).

QUALITY OF LIFE

The quality of life was assessed with the use of the EORTC QLQ-C30 instrument. When responses at base line were compared with responses during 12 weeks of eculizumab treatment, there were significant improvements in the domains of global health status ($P=0.02$), physical functioning ($P<0.001$), emotional functioning ($P<0.001$), cognitive functioning ($P=0.002$), fatigue ($P<0.001$), dyspnea ($P=0.002$), and insomnia ($P=0.049$) (Table 2).

DISCUSSION

Patients with PNH have chronic, often disabling symptoms of fatigue and intermittent episodes of dysphagia, abdominal pain, and hemoglobinuria. These symptoms are thought to be related to the intravascular destruction of PNH type III erythrocytes, which are deficient in complement inhibitors, by autologous complement. The hemolytic anemia frequently renders the patients transfusion-dependent. In addition, patients have an extremely high risk of potentially life-threatening thrombosis, particularly thrombosis of the hepatic and cerebral veins. Approximately 50 percent of patients with PNH die of the disease; the median duration of survival after diagnosis is 10 years.[1]

We found that the defect in the membrane-bound inhibitor of terminal complement components in PNH was ameliorated by the administration of eculizumab. This antibody specifically prevents cleavage of C5, which is necessary for assembly of the membrane-attack complex. Blockade of terminal complement components presumably prolongs the survival of type III erythrocytes (since there was no simultaneous increase in reticulocytes), which are highly sensitive to lysis by complement, thereby increasing the proportion of these cells in the blood and reducing signs of hemolysis in most patients. In some patients, the percentage of type III erythrocytes increased to more than 80 percent of the total erythrocyte population. This interpretation of the mechanism of action of eculizumab is consistent with the report of an asymptomatic patient with PNH who had more than 80 percent type III erythrocytes and a concomitant deficiency in the terminal complement protein C9.[20] Thus, inhibition of the assembly of C5b–C9 by an antibody or by a congenital deficiency of a terminal component of the complement system can protect type III erythrocytes from complement-mediated lysis.

The long-term effects of protecting PNH type III erythrocytes from complement are not known. For example, will removing the negative pressure

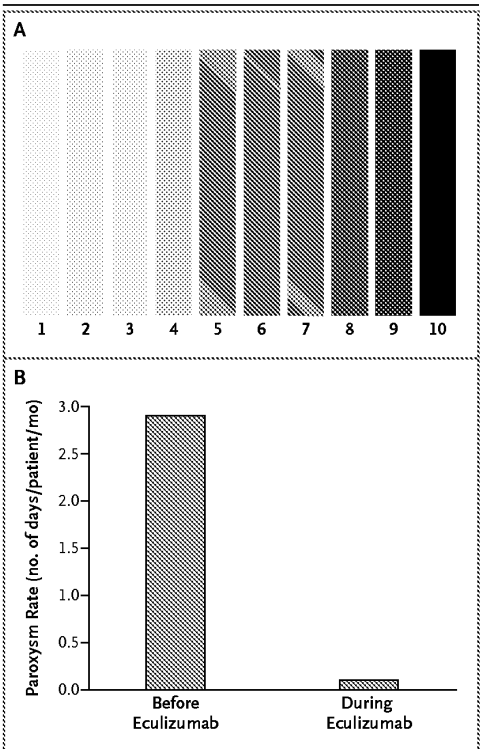

Figure 4. **Incidence of Paroxysms during Eculizumab Treatment.**

Panel A shows a urine color scale devised to monitor the incidence of paroxysms of hemoglobinuria in patients with paroxysmal nocturnal hemoglobinuria before and during treatment. A paroxysm was prospectively defined as a urine score of 6 or greater in this study. Panel B depicts the change in the paroxysm rate (defined as the mean number of days in paroxysm per patient per month) in nine patients 1 month before and during 12 weeks of eculizumab therapy. Pretreatment data on paroxysms were inadvertently not collected for two patients, and these two were therefore excluded from the analysis.

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Table 2. **Change in the Quality of Life during Eculizumab Treatment.***

Domain	Mean Base-Line Score†	Change from Base Line‡§	P Value§
Global health status	56.1	13.7	0.02
Physical functioning	70.9	13.0	<0.001
Emotional functioning	70.5	12.7	<0.001
Cognitive functioning	77.3	11.8	0.002
Fatigue	47.5	−15.3	<0.001
Dyspnea	39.4	−12.4	0.002
Insomnia	30.3	−10.8	0.049

* The quality of life was assessed with the European Organization for Research and Treatment of Cancer QLQ-C30 instrument.
† Numbers represent mean values of linearly transformed scores.
‡ Values for change from base line represent least-square means. A positive value indicates an improvement in the score for global health status, physical functioning, emotional functioning, and cognitive functioning, whereas a negative value indicates an improvement in the score for fatigue, dyspnea, and insomnia.
§ Values are from a mixed analysis-of-covariance model with visit as a fixed effect, patient as a random effect, and base line as a covariate.

on type III hematopoietic cells alter the rate of expansion of the PNH clone? What might occur in a patient with an increased population of PNH type III erythrocytes if treatment with eculizumab is stopped? Two of the patients who entered the eculizumab extension study had transient breakthroughs (lasting two to three days) in complement blockade until the dosing interval was adjusted. Both patients had hemoglobinuria with mild symptoms, but the episodes were not life threatening and were easily managed (data not shown). Definitive answers to these questions will require further study.

In this trial, lactate dehydrogenase levels declined rapidly and remained reduced as long as the serum level of eculizumab exceeded 35 μg per milliliter. The importance of maintaining this level of antibody was demonstrated in a single patient, in whom the eculizumab level transiently dropped below 35 μg per milliliter at week 12, resulting in a return of serum complement activity and an increase in lactate dehydrogenase levels. Subsequent administration of eculizumab reestablished complement blockade and rapidly reduced lactate dehydrogenase levels. Interestingly, lactate dehydrogenase levels were reduced in most patients to just above the upper limit of normal. The slightly elevated levels of this enzyme during treatment with eculizumab could reflect persistent, low-level C3b-mediated extravascular hemolysis or, possibly, undefined mechanisms of hemolysis that are unrelated to complement.

We also found that eculizumab treatment significantly reduced transfusion requirements, even though the levels of hemoglobin did not change significantly. However, the hemoglobin level in an individual patient before study entry was artificially maintained as a result of the transfusion of normal red cells. The transfused red cells survive far longer than PNH cells. Therefore, the stabilization of hemoglobin levels with a reduced need or no need for transfusion is a result of the protection of PNH red cells from complement-mediated lysis by eculizumab.

The decrease in transfusion requirements was most apparent in the six patients without a clinically significant degree of bone marrow failure (as defined by a normal platelet count). All but one of these patients were transfusion-independent during the study and remained so during an extension study. The remaining patient received a single 3-unit transfusion during the study, as compared with the receipt of 55 units in the 12 months preceding enrollment.

There was a rapid improvement in the quality of life during eculizumab therapy, as measured by the EORTC QLQ-C30. These clinical observations support the hypothesis that many of the important coexisting clinical conditions in patients with PNH are directly related to chronic and acute episodes of hemolysis, possibly through the scavenging of nitric oxide by plasma free hemoglobin.[21-25]

Eculizumab was safe and well tolerated during this open-label pilot study. The adverse events reported by patients were similar in type and frequency to those reported with either eculizumab or placebo in other controlled trials. All patients are currently participating in a one-year extension study in which the drug continues to be well tolerated. Furthermore, the rates of intravascular hemolysis, as measured by lactate dehydrogenase levels and hemoglobinuria, remain reduced in all patients, with 5 of 11 patients having been transfusion-independent for at least one year since starting eculizumab treatment.

The *PIG-A* mutation in patients with PNH causes deficiencies in the membrane-bound complement inhibitors CD55 and CD59, resulting in intravascular hemolysis.[2,3] However, patients who have genetic deficiencies in the surface expression of CD55 (Inab phenotype) with normal levels of CD59 have no clinical signs of hemolysis.[8,9] Conversely, a pa-

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tient with a genetic deficiency in the expression of CD59 but normal levels of CD55 had symptoms indistinguishable from those of PNH.[10,11] Therefore, the somatic mutation in the *PIG-A* gene that causes the deficiency of the membrane-bound terminal complement inhibitor CD59 is critical to the pathogenesis of PNH. We found that terminal complement inhibition with eculizumab ameliorated the untoward effects of this deficiency.

In summary, eculizumab appears to enhance the survival of type III PNH erythrocytes, improving the quality of life and reducing the extent of hemolysis, hemoglobinuria (the clinical hallmark of PNH), and the need for blood transfusions in patients with PNH. This study confirms that terminal complement activation is the key mediator of erythrocyte destruction in PNH.

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Eculizumab Alexion

Mariana Kaplan

Address
University of Michigan Medical Center
Division of Rheumatology
Int MED-Rheumatology
3918 TC
0358
Ann Arbor
MI 48109-1065
USA
Email: makaplan@umich.edu

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Eculizumab (5G1.1), a humanized monoclonal antibody that prevents the cleavage of human complement component C5 into its pro-inflammatory components, is under development by Alexion as a potential treatment for several chronic inflammatory diseases, including rheumatoid arthritis (RA) and nephritis [130673], [292328]. In January 2002, a phase IIb trial was initiated for RA [437814]. This trial was ongoing in April 2002, at which time, eculizumab was also in phase II trials for the treatment of membranous nephritis and lupus nephritis, and in earlier stage clinical trials for dermatomyositis and pemphigoid [446577]. The company is also developing a single-chain version of this antibody, pexelizumab, for use in acute cardiovascular indications [188760].

In October 2000, eculizumab was granted Orphan Drug status by the FDA for the treatment of dermatomyositis [385057]. In February 2002, the product received Orphan Drug designation for its use in patients with membranous nephritis [440583].

In September 2001, analysts at US Bancorp Piper Jaffray predicted eculizumab's launch for dermatomyositis and pemphigus in 2003, RA and nephritis in 2004, and chronic heart failure (CHF) after 2006 [426537]. In March 2002, analysts at US Bancorp Piper Jaffray predicted that the product would have peak worldwide sales in RA of US $175 million and US $400 million for nephritis. Sales for the RA indication are predicted to reach US $35 million in 2006, rising to US $110 million in 2008, and US $10 million in 2006, rising to US $50 million in 2008, in the US and the rest of the world, respectively. For the nephritis/other indication(s), sales are pegged at US $50 million in 2006, rising to US $200 million in 2008, and US $15 million in 2006, rising to US $100 million in 2008, again in the US and the rest of the world, respectively [446992].

Originator Alexion Pharmaceuticals Inc

Status Phase II Clinical

Indication Dermatological disease, Glomerulonephritis, Nephritis, Pemphigus, Psoriasis, Rheumatoid arthritis, Systemic lupus erythematosus

Action Complement cascade inhibitor

Biotechnology Monoclonal antibody, humanized

Synonyms 5G1.1, h5G1.1, C5 complement inhibitor (Alexion), h5G1.1scFv

CAS 5G1.1
Registry No: 339087-76-2

Introduction

The complement system comprises more than 30 human proteins in plasma and on cell surfaces that interact in a cascade sequence to mediate a variety of inflammatory events. These events include the opsonization of particles for phagocytosis, leukocyte activation and assembly of the membrane attack complex (MAC) [435653], [435654], [435718], [445228]. The system is responsible for killing and removing organisms, cells and cell components from the circulation which are recognized as foreign. In addition to host defense against bacterial agents, complement is involved in the disposal of immune complexes and the products of inflammatory injury, and serves as an interface between innate and adaptive immunity [435653].

Several complement proteins are cleaved during activation of the complement system and three pathways of activation have been described; the classical, the alternative and the lectin pathways [435653]. The pathways leading to the cleavage of C3 are triggered enzyme cascades. Downstream in these pathways, C3 is cleaved into C3a and C3b. C3a is released while C3b forms C5 convertases, which in turn cleave C5 to C5a and C5b. Following complement activation, pro-inflammatory peptides, such as the anaphylotoxins C3a and C5a, are generated and C5b-9 (MAC) is formed. Assembly of the MAC from the terminal components (C5 to C9) of the cascade leads to membrane damage. In addition, complement activation products, especially the anaphylotoxins, elicit a number of biological effects, including chemotaxis of leukocytes, degranulation of phagocytic cells, mast cells and basophils, smooth muscle contraction and the increase of vascular permeability [435653], [445228], [445736].

Under normal circumstances, the regulatory mechanisms of complement are balanced, so that the activation of complement occurs on the surface of invading microorganisms and the deposition of complement on normal cells and tissues is usually limited [435653], [445228]. If the mechanisms that regulate this balance are abnormal, the complement system might then cause tissue injury [435656]. Excessive or inappropriate activation of the complement system can lead to harmful and life-threatening consequences which are secondary to severe inflammatory tissue destruction. These consequences can manifest clinically in a variety of ways, including multiple organ failure, hyperacute graft rejection and septic shock [435656]. Inappropriate complement activation and its deposition on host cells can also lead to direct complement-mediated cell lysis of target structures, or indirectly to tissue destruction due to the generation of powerful mediators of inflammation [435653], [445228].

Complement plays an important role in the pathogenesis of many autoimmune and inflammatory diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) [435694], [445236] and dermatomyositis [445242]. It is also responsible for disease states associated

with bio-incompatibility, including transplant rejection [445249]. In addition, complement activation plays an important role in the pathogenesis of cardiac and intestinal ischemia/reperfusion injury [445251]. Other conditions in which complement activation has been proposed to play a role include stroke [445254], multiple sclerosis [445384], pancreatitis [445255], psoriasis [445272], Alzheimer's disease [445278] and prion-induced diseases [445286].

Therapeutic inhibition of complement is, therefore, potentially important in protecting against the development of certain diseases and it has been shown to be useful in animal models of sepsis, intestinal ischemia/reperfusion injury, myocardial reperfusion injury, nephritis, arthritis and graft rejection.

Several complement inhibitors are currently under investigation [212463], [225318], [281110], [435656], [435722], [435723], [435725], [436916]. Eculizumab (5G1.1), under development by Alexion Pharmaceuticals Inc, is a humanized C5 inhibitory monoclonal antibody (mAb) that prevents the cleavage of human complement component C5 into its pro-inflammatory components and targets the complement components, C5a and C5b-9 [445382]. As mentioned, the early steps of the complement cascade play an important role in the removal of infectious agents and several normal functions of the immune system. The rationale of specifically blocking C5 is that the normal upstream disease-preventing functions of complement remain intact, while the production of the abnormal downstream disease-causing actions of complement are blocked. This antibody, therefore, potentially avoids the disruption of antibacterial-protective mechanisms, which are believed to be mediated primarily through C3b [438165].

Eculizumab is undergoing phase II clinical trials for a variety of chronic inflammatory conditions, and is being developed as a potential treatment for RA, SLE and nephritis [190673], [292328], [412090]. The drug is currently in phase II trials for nephritis and has completed phase I/II trials in SLE and phase II trials in RA [328001], [335241], [335995], [396672]. Eculizumab is also currently undergoing phase I trials for psoriasis and pemphigus [384948], and has been granted Orphan Drug status by the FDA for the treatment of dermatomyositis [385057], and membranous nephritis [440583].

Alexion has also developed a short-acting C5 complement inhibitor that is a humanized single chain mAb fragment. This compound is known as pexelizumab (formerly 5G1.1-SC) and it is being tested for the treatment of acute cardiovascular diseases associated with complement activation, including acute myocardial infarction (MI), unstable angina and revascularization/reperfusion therapies. This drug was designed to have the advantage of enhanced tissue penetration, desirable for effective treatment of these acute life-threatening conditions [188760]. Pexelizumab and eculizumab are currently in at least eight clinical development programs [435963]. A phase III clinical study with pexelizumab in cardiopulmonary bypass patients has been initiated. In addition, two large phase II studies with pexelizumab in acute myocardial infarction (MI) patients are under way. Since pexelizumab has recently been evaluated, this review will focus primarily on eculizumab.

Synthesis and SAR

Humanized 5G1.1, Fab and scFv molecules were produced by grafting the complementary determining regions of 5G1.1 on to human framework regions [258884]. Competitive ELISA analysis indicated that no framework changes were required in the humanized variable regions for retention of high affinity binding to C5, even at framework positions predicted by computer modeling to influence CDR canonical structure. The humanized Fab and scFv molecules blocked complement-mediated lysis of chicken erythrocytes and porcine aortic endothelial cells in a dose-dependent fashion. Complete complement inhibition occurred at 3-fold molar excess, relative to the human C5 concentration. Humanized 5G1.1 scFv also effectively blocked C5a generation. In addition, intact humanized 5G1.1 antibody blocked human complement lytic activity at concentrations identical to the original murine monoclonal antibody, indicating that humanized 5G1.1 and its derivatives retained both the affinity and blocking functions of murine 5G1.1 antibody [258883], [258884]. 5G1.1 is supplied as a sterile, non-pyrogenic solution (2 mg/ml) for iv injection [212467], [258884] and it has picomolar affinity for a sequence within human C5. 5G1.1 can be administered either intramuscularly or subcutaneously, allowing self-administration [438165].

Pharmacology

Heart disease

There is a growing body of evidence pointing to a substantial disease-promoting role of complement components in MI and unstable angina [445289]. Activation of the complement cascade in MI patients is shown by elevated levels of activated complement byproducts in the blood of patients during acute MI and by the deposition of activated complement components in damaged heart tissue [445382]. Interestingly, terminal complement components play a key role in the apoptotic process in heart tissue during MI [241313], and blockade of the complement cascade at C5 has been shown to substantially reduce myocardial apoptosis and tissue damage in rats [241313]. Complement activation was also found in patients undergoing cardiopulmonary bypass (CBP) [445311]. This finding was associated with increased patient morbidity, including MI, stroke, cognitive dysfunction and blood loss [445311].

Studies in primates demonstrated that inhibition of complement activation reduces heart damage associated with coronary ischemia and reperfusion in preclinical models. Furthermore, treatment of hypoxic/reoxygenated human umbilical vein endothelial cells (HUVECs) with h5G1.1 scFv attenuated C5b-9 deposition, NFκB translocation and vascular cell adhesion molecule (VCAM)-1 expression [363137].

Intestinal and lung ischemia-reperfusion

A recent study reported that anti-C5a therapy significantly improved intestinal ischemia-reperfusion tissue injury as well as lung injury in rats [445313]. These results suggested that C5 inhibition could also limit the complaints in the broader general surgery population as well as in patients with ulcerative colitis [357827] Interestingly, C5 inhibition was associated with a significant reduction in tumor necrosis factor (TNF)α levels [357827].

Inflammatory arthritis

Animals deficient in the complement component C5 are not susceptible to the onset of active arthritis [363495]. In the absence of C5 cleavage, the presence of TNFα is not sufficient to promote arthritis. A recent study reported that administration of anti-C5 mAb ameliorates the established collagen-induced arthritis in mice and rats [190673], [212456]. Systemic administration of the anti-C5 mAb effectively inhibited terminal complement activation *in vivo* and prevented the onset of arthritis in immunized animals. In addition, anti-C5 mAb treatment was also highly effective in ameliorating established disease. These results suggested a critical role for activated terminal complement components, not only in the induction, but also in the progression of collagen-induced arthritis [190673], [212456].

Renal disease and lupus

A C5 inhibitor was administered in both an acute and rapid progressive glomerulonephritis (RPGN) model that normally leads to crescentic changes, the most common histological lesion observed in lupus nephritis. The inhibitor was also administered to the NZB/NZW F1 lupus-prone mouse model. C5 inhibition prevented histological and clinical development of nephritis in the RPGN model and C5 inhibitor administration in the lupus-prone mouse model reduced histologic and biochemical evidence of kidney disease. Survival at 10 months was markedly improved from 5% in placebo-treated lupus-prone mice to 85% in C5 inhibitor-treated lupus-prone mice [190673], [223608], [280073].

Hematological diseases

C5 inhibition has also been shown to prevent platelet loss in a model of idiopathic thrombocytopenic purpura (ITP) [392060].

Metabolism

5G1.1 blocks complement activity for 1 to 2 weeks after a single dose [438165].

Toxicity

No toxicity has been reported in humans [228105], [392382] or rhesus monkeys [212462]. Interestingly, a recent study reported that C5 plays an important role in liver regeneration, strongly implicating the complement system as a crucial regulatory component of hepatic homeostasis. C5-deficient mice show severely defective liver regeneration and persistent parenchymal necrosis after exposure to CC_{l_4}. In addition, these mice show marked delays in the re-entry of hepatocytes into the cell cycle (S phase) and diminished mitotic activity. Reconstitution of C5-deficient mice with murine C5 or C5a significantly restores hepatocyte regeneration after toxic injury. Furthermore, blockade of the C5a receptor abrogates the ability of hepatocytes to proliferate in response to liver injury [445317]. The role of eculizumab on hepatic regeneration is unclear at this point.

C5 is also known to mediate chemotactic and activation events that are the basis for granulomatous responses during murine tuberculosis. Mycobacterium tuberculosis-infected mice with natural deficiency in C5 are unable to develop productive granulomatous responses, and are impaired in limiting organism growth within the lung [445321].

Clinical Development

Phase I

Rheumatoid arthritis

A phase I/II, multicenter, double-blind, placebo-controlled, dose-escalating trial tested the role of eculizumab in ameliorating RA. The trial was designed to gather clinical data on the safety profile and biological effects of a single administration of eculizumab in RA. The preliminary analysis demonstrated that the drug was well tolerated and was associated with a significant reduction in disease activity in these patients [445382]. The safety and biological activity of the antibody was examined in 40 patients with mild-to-moderate RA, each of whom received a single dose of eculizumab (0.1 to 8 mg/kg). The drug was safe and well tolerated, and showed no detectable immunogenicity in the population studied. Furthermore, a single dose of eculizumab potently and rapidly blocked complement activity in a dose-dependent fashion for up to 2 weeks [353141]. Complement inhibition after a single dose was associated with a beneficial clinical effect, with the highest dose (8 mg/kg; n = 6) resulting in a significant 30% reduction in C-reactive protein levels, compared to placebo-treated patients in which C-reactive protein increased by 24%. Eculizumab was also associated with a trend in reductions in other measurement of disease activity, including tender joint count, swollen joint count, patients' global assessment of disease and patients' global assessment of pain [291142], [305950], [322159], [335241], [347482], [335995].

Systemic lupus erythematosus

Alexion has completed preliminary analysis of the phase I study of eculizumab in SLE. The trial was designed to gather data on pharmacodynamics, safety and biological effects of a single administration of the drug in SLE patients. Eculizumab was well tolerated and its administration was associated with significant reduction in the incidence of proteinuria. The phase I, double-blind, placebo-controlled, dose-escalating trial examined the safety and biological activity of eculizumab in 24 patients with mild SLE, each of whom received a single dose of the antibody (0.1 to 8 mg/kg). There was no detectable immunogenicity in the patients studied and a single dose of antibody blocked complement activity in patients for up to 2 weeks. Administration of a single dose of 8 mg/kg was associated with significant decrease in proteinuria [292328], [328001].

Dermatomyositis

In 2001, Alexion completed a 2-month, phase I, pilot safety trial of eculizumab in 13 dermatomyositis patients with persistent dermatomyositis, undergoing concomitant treatment with moderate doses of methotrexate or steroids. The patients were evaluated in either a placebo (n = 3) or a single drug treatment arm (n = 10). Drug treatment consisted of eculizumab (8 mg/kg/week iv) for 3 weeks and then 8 mg/kg every 2 weeks for up to 2 months. The patients were evaluated after 2 months of treatment for safety and for trends in clinical improvement. In this placebo-controlled, multicenter trial, the drug was safe and well tolerated and associated with an improvement in skin rash. Exploratory clinical measurements included clinical and laboratory assessments of skin rash and muscle strength. There were consistent trends in improvements with drug administration in subjective and objective

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measurements of skin rash during the 2-month trial. While there was little baseline skin inflammation in the placebo group, a majority of drug-treated patients who completed the trial experienced an improvement of ≥ 50% in their skin rash score [354030], [434351], [435888].

Pemphigoid

Published observations have shown that clinical improvement of pemphigoid is associated with reduced levels of complement activation in the previously affected skin, further supporting the rationale for testing the C5 inhibitor in this disease. Phase I trials of eculizumab in pemphigoid syndrome are ongoing [361798], [426537], [435888], but no data are currently available.

Psoriasis

A single-center, double-blind, placebo-controlled study was designed to evaluate the safety profile and clinical effects of eculizumab in severe psoriasis [352110]. In June 2001, Alexion completed a phase I 'pilot' safety trial involving 40 psoriasis patients, which showed that the drug was well tolerated. Drug administration did not influence the clinical outcome as measured by Psoriasis Area and Severity Index (PASI) score, although favorable trends in certain measures of disease activity were observed. Drug administration dose-dependently blocked hemolytic activity in the blood of treated patients and dose-dependently reduced deposition of activated terminal complement in psoriatic plaques [412091].

Phase II

Rheumatoid arthritis

In a phase II trial in 209 patients, eculizumab administration appeared to be safe and well tolerated and the adverse event profile was comparable to placebo. Patients were treated with placebo, eculizumab (8 mg/kg iv) once per week for 4 weeks and then once every month (induction/monthly group); eculizumab (8 mg/kg iv) once per week for 4 weeks followed by once every 2 weeks (induction/biweekly group); or, eculizumab (8 mg/kg iv) once every 2 weeks (biweekly group). The results after 3 months of treatment showed that the induction/monthly group met the primary endpoint of the trial (improvement in American College of Rheumatology response criteria score (ACR) 20 after 3 months of treatment), while the induction/biweekly and biweekly groups did not statistically meet the endpoint. The ACR20 response in the induction/monthly group was 44% as compared to 18% ACR20 in the placebo group, by per protocol analysis. Both induction/monthly and induction/biweekly groups also met the secondary endpoint of changes in C-reactive protein after 3 months of therapy [429348].

In patients with elevated C5b-9 levels over 200 ng/ml, the ACR20 at 3 months of treatment were dose-dependent. The ACR20 results obtained in patients with elevated baseline C5b-9 levels were: placebo (9%); biweekly (33%); induction/monthly (57%); and induction/biweekly (50%). Further data from this trial are to be released following unblinding [397163], [429348].

Alexion has also started a phase IIb trial in RA patients. The trial is designed to assess the safety and efficacy of eculizumab and to confirm the most efficacious dose regimen. The trial will consist of approximately 300 patients with mild-to-moderate disease who are being treated concomitantly with disease-modifying antirheumatic drugs such as methotrexate or leflunomide. The trial will consist of three treatment arms; patients will be treated with placebo; eculizumab (8 mg/kg/week iv) for 4 weeks and then once every month; or eculizumab (8 mg/kg/week) for 4 weeks and then bimonthly. The patients will be evaluated after a 6-month drug phase for safety and efficacy, and the primary efficacy endpoint will be the ACR20 score [437814].

Systemic lupus erythematosus and nephritis

In August 1999, Alexion initiated a phase II study with eculizumab in lupus patients suffering from membranous nephritis [335995]. The trial is expected to enroll approximately 40 lupus nephritis patients at four clinical sites in the US and has been designed to test the safety and biological efficacy of chronic administration of eculizumab for up to 6 months [412090]. In September 2001, Alexion expected phase II results to be available in mid-2002 [426537].

Side Effects and Contraindications

In clinical trials to date, the drug was well tolerated and showed no detectable immunogenicity in the patients studied. No major adverse reactions have been reported. In the dermatomyositis trial, the most common side effects were headache and rash, and it appeared that these side effects were comparable to the placebo group. In the psoriasis trial, the most common side effects were headaches and unspecified pain. The most common side effects in the RA trial were comparable to placebo and included diarrhea and headaches [429348], [434351].

Patent Commentary

In March 2002, Alexion was issued US-06355245 entitled 'C5-specific antibodies for the treatment of inflammatory diseases.' The patent covers the composition of Alexion's lead drug candidates, eculizumab and pexelizumab, and other C5-binding anti-inflammatory antibodies [443717]. WO-09529697, entitled 'methods and compositions for the treatment of glomerulonephritis and other inflammatory diseases' describes a method for the possible treatment of glomerular inflammation and enlargement, involving the administration of low dosages of anti-C5 antibodies. WO-09609043 describing a method for the treatment of established joint inflammation is claimed in which a C5 blocker is administered.

Current Opinion

C5 inhibitors appear to intervene at a point that allows preservation of the anti-inflammatory and antibacterial responses at the C3 level, while conceivably inhibiting the downstream disease-causing actions. Selective suppression of this immune response could certainly provide a significant therapeutic advantage compared to existing therapies. Eculizumab blocks the production of harmful complement components and appears to be promising for the treatment of various inflammatory diseases, including RA, SLE and a variety of conditions with skin involvement, including dermatomyositis, pemphigoid and psoriasis. There are other conditions in which C5a seems to play an important role, including asthma and HIV infection, and these conditions might potentially become therapeutic targets in which C5a inhibition could be useful [445327], [445336].

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Since complement activation has both positive and negative effects, the risk associated with complement inhibition is probably not negligible and long term studies will be necessary to assess possible risks. In particular, a theoretical concern is the role that C5 plays in hepatic regeneration and in combating granulomatous infections such as tuberculosis. Long term studies will determine whether C5 inhibition could have deleterious effects in these processes.

So far, limited animal and human studies have shown that eculizumab inhibits complement in a dose-dependent manner, is well tolerated and has no significant side effects. The results have been encouraging so far but more clinical trials are needed. There are still concerns that this drug does not block activation of the early stages of the complement system. This inhibitor allows the generation of C3a. While C3a is considered a less potent inflammatory molecule than C5a, the C3a receptor tissue expression appears to be much broader than originally expected [445349], [445352], and recent studies have shown that C3a can induce the production of inflammatory cytokines in a fashion similar to C5a [445354]. C3a could, in theory, play a significant role in inflammatory conditions and acute-phase response even after C5 inhibition. Nevertheless, if clinical trials prove successful, this drug as well as pexelizumab, could have a broad range of applications where complement-mediated inflammation contributes to disease pathology. There are currently no biological therapies on the market with the actions and specificity of these drugs and eculizumab could have a very strong therapeutic potential.

Development history

Developer	Country	Status	Indication	Date	Reference
Alexion Pharmaceuticals Inc	US	Phase II clinical	Rheumatoid arthritis	09-JUL-98	291142
Alexion Pharmaceuticals Inc	US	Phase II clinical	Nephritis	12-AUG-99	335995
Alexion Pharmaceuticals Inc	US	Phase I clinical	Psoriasis	06-OCT-00	384948
Alexion Pharmaceuticals Inc	US	Phase I clinical	Dermatological disease	06-OCT-00	384948
Alexion Pharmaceuticals Inc	US	Phase I clinical	Pemphigus	06-OCT-00	384948
Alexion Pharmaceuticals Inc	US	Preclinical	Glomerulonephritis	28-JUL-97	190673
Alexion Pharmaceuticals Inc	US	NDR	Systemic lupus erythematosus	11-APR-02	-

Literature classifications

Biology

Study Type	Effect Studied	Experimental Model	Result	Reference
In vivo	Anti-arthritic effect.	Collagen-induced arthritis in mice and rats.	An anti-C5 antibody both prevented the onset of disease and reduced established disease.	190673 212456
In vivo	Reduction in evidence of kidney disease.	NZB/NZW F1 lupus-prone mouse model.	An anti-C5 antibody reduced both the histological and biochemical signs of nephritis, and increased survival to 85% at 10 months.	232606

Metabolism

Effect Studied	Model Used	Result	Reference
Pharmacokinetics.	Complement activity.	A single dose of eculizumab blocks complement activity for 1 to 2 weeks.	436165

Clinical

Effect Studied	Model Used	Result	Reference
Safety and efficacy.	Phase I trial in 40 psoriasis patients.	Eculizumab was well tolerated and dose-dependently reduced complement activity in patients. Favorable trends were observed in PASI, although these were not significant.	412091
Safety and efficacy.	Phase I trial in 13 patients with persistent dermatomyositis.	Eculizumab (6 mg/kg/week iv) for 5 weeks, then every 2 weeks for up to 2 months, was safe and well tolerated. Drug treatment reduced skin rash score by ≥ 50%.	434351
Safety and efficacy.	Double-blind, placebo-controlled, phase I, dose-escalating trial in 24 patients with mild SLE.	Eculizumab (0.1 to 6 mg/kg) was safe and well tolerated, and no detectable immunogenicity was observed. The highest dose significantly reduced proteinuria.	328001
Safety and efficacy.	Phase II trial in 209 RA patients.	Eculizumab produced ACR20 in 44% of the patients treated. Adverse effects were similar to placebo, with nausea and diarrhea being the most common complaints.	429348
Safety and efficacy.	Phase II trial in RA patients in whom the C5b-9 levels were > 200 ng/ml.	A total of 57% of patients achieved ACR20 when treated with induction/monthly treatment of eculizumab.	429348

Associated patent

Title Method for reducing immune and hemostatic dysfunctions during extracorporeal circulation.

Assignee Alexion Pharm Inc

Publication WO-09526540 28-SEP-95

Priority US-00217391 23-MAR-94

Inventors Rollins SA, Smith BR, Squinto SP.

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New approaches to the treatment of glomerular diseases

GB Appel[1], M Waldman[1] and J Radhakrishnan[1]

[1]*Division of Nephrology, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York, USA*

The treatment of primary glomerular diseases has previously focused on relatively toxic immunosuppressive regimens. We are now seeing a paradigm shift from these toxic 'nonspecific' therapies to selective immunomodulating and immunosuppressive regimens. In this article, the experience with these newer agents including mycophenolate, rituximab, sirolimus, calcineurin inhibitors, and eculizumab are described.

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KEYWORDS: glomerular disease; mycophenolate; rituximab; sirolimus; calcineurin inhibitors; eculizumab

Correspondence: *GB Appel, Division of Nephrology, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York, USA. E-mail: gba2@columbia.edu*

In recent years, the therapy of glomerular diseases has dramatically changed. Certain 'nonspecific' interventions appear to be beneficial in almost all patients with renal diseases. These include optimizing blood pressure control to a level of no greater than 130/80 mm Hg and use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, either alone, or in combination. Recent data also suggests that HMG-CoA reductase inhibitors may decrease proteinuria and slow progression of renal disease. Although the data for dietary therapy remains more controversial, many clinicians utilize moderate protein restriction, low cholesterol/saturated fat, and low salt diets in their glomerular disease patients.

Despite the significant benefits of these non-immune modulating therapies, we are still in need of better immune-modulating drugs to treat glomerular diseases. In part, this is to control refractory disease, and in part to prevent relapses and flares. Patients who experience complete (or even partial) sustained remissions of proteinuria have less glomerular sclerosis, interstitial fibrosis, and slower progression to renal failure. Finally, there is a need to design effective immuno-suppressive strategies while avoiding the toxicities of conventional global immunosuppression.

The use of newer immune-modulating agents in a variety of glomerular disease remains to be defined. This is true despite some well-designed controlled randomized trials. In this manuscript, we review some of the recent studies examining the use of newer immunosuppressants in common glomerular diseases, including lupus nephritis, immunoglobulin A (IgA) nephropathy, focal glomerulo-sclerosis, and membranous nephropathy. We emphasize the value and shortcomings of these studies and describe new on-going trials.

LUPUS NEPHRITIS

In lupus nephritis (LN), advances in immunosuppressive treatment have occurred in recent years due to results from large, multicenter randomized controlled trials (RCT). Until recently, the standard treatment of severe LN consisted of 6 monthly pulses of IV cyclophosphamide (CYC) (0.5–1 g/m^2) followed by follow-up CYC pulses every 3 months based upon trials from the National Institutes of Health.[1–4] Some clinicians gave concomitant IV methylprednisolone based on data suggesting improved long-term renal survival without

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additional toxicity. Despite considerable efficacy, this regimen was associated with short- and long-term adverse effects, and some patients failed to achieve remission or relapsed after treatment. Thus, major efforts have been taken to develop alternate immunosuppressive regimens that are equally effective but less toxic. Strategies include minimizing use of CYC with lower dosages, use of sequential therapies with different immunosuppressive agents and avoidance of CYC entirely with alternate agents. Although no specific regimen has become the new standard of care for LN, recent studies lend strong support to the use of alternate treatment regimens for both induction and maintenance regiments for proliferative LN.

The Euro Lupus Trial by Houssiau *et al.*[5] compared low-dose to conventional high-dose IV CYC as induction therapy for severe LN. Ninety patients, of whom 85% were white with class IV LN, were randomized to either standard doses CYC (6 monthly IV pulses followed by two quarterly pulses) or low-dose CYC (fixed pulses of 500 mg biweekly $\times$ 6) followed by maintenance with azathioprine (AZA). There was no significant difference in renal remission with high-dose CYC (54%) and low-dose CYC (71%) and renal flares occurred in 29 and 27%, respectively. There was a trend towards more infections with high-dose CYC. This trial suggests that a shorter course of lower dose CYC for induction followed by AZA may be an equally effective and less-toxic regimen. However, it is unclear whether these results are applicable to all high-risk patients as the study included few black patients, a population known to be a greater risk of progressive disease.[6,7]

Several major controlled trials have demonstrated the role of mycophenolate mofetil (MMF) as a promising therapeutic alternative for treatment of severe LN. A trial by Chan *et al.*[8] randomized 42 Chinese patients with DPLN to either MMF (induction with 2 g/day $\times$ 6 months, followed by maintenance with 1 g/day for 6 months) or 6 months induction with oral CYC (2.5 mg/kg/day) followed by maintenance with AZA for 6 months. At the end of 12 months, MMF and CYC were found to have equivalent efficacy with no differences in complete and partial remissions or relapses. Extended observation over 5 years with addition of 22 patients confirmed the long-term benefits of MMF with similar rate of relapse and relapse-free survival in both groups.[9] Adverse events including leukopenia, amenorrhea, and infections were more frequent in the CYC group and two deaths occurred in this group. This suggests a role for MMF in induction therapy for many Class IV lupus patients. Similar results have been confirmed by another trial of 46 Chinese patients treated with either MMF or pulse IV CYC for 6 months, which showed greater reductions in proteinuria, hematuria, anti-DNA antibody titers, and greater improvement on repeat renal biopsy in patients treated with MMF.[10]

A recently published multicenter RCT by Ginzler *et al.*[11] evaluated the efficacy of MMF in 140 patients with proliferative LN. In this study, 56% of the patients were African Americans with active urinary sediment, significant proteinuria, and active lupus serologies, thus representing a high-risk population. Patients were randomized to induction therapy with either standard 6 monthly pulses of IV CYC or MMF (target dose 3 g/day) in conjunction with a fixed tapering course of corticosteroids. The study allowed cross-over at 3 months for treatment failures. At six months, there were more complete remissions and complete plus partial remissions in the MMF arm. Side effect profile was also better in the MMF group with fewer severe infections. At median follow up of 3 years, there was no difference between treatment arms in number of patients with renal failure, end-stage renal disease or mortality, although all tended to be lower with MMF. A major limitation of the study is that it lacks long-term follow up. Furthermore, it is unclear if patients with more severe LN with crescents and necrosis will have a similar favorable response to MMF. Nevertheless, this study adds significantly to our experience with MMF in high risk, non-white patients. A large multicenter international randomized trial is now underway to further evaluate and compare induction therapy with either MMF or IV CYC (Aspreva Lupus Management Study) (abstract; Appel G *et al.* *J Am Soc Nephrol* 2005; **16**: 528A)

MMF was evaluated as maintenance therapy for proliferative LN in a study which randomized 59 patients to receive either MMF, AZA, or quarterly IV CYC after induction with standard monthly IV CYC pulses.[12] Fewer patients treated with AZA and MMF reached the primary end points of death and chronic renal failure (CRF) compared to the CYC group. Relapse-free survival was also higher with MMF and AZA. There was increased mortality in the IV CYC group. Hospitalizations, amenorrhea, infections, and gastrointestinal side effects were significantly lower in the MMF and AZA groups. A RCT (MAINTAIN Nephritis Trial) conducted by the European Working Party on Systemic Lupus Erythematosus is currently underway to compare the efficacy of MMF and AZA as maintenance treatment for proliferative LN following induction therapy with a short course of IV CYC.

Rituximab, a chimeric monoclonal antibody directed against the pan B-cell marker CD20 has been used to treat LN in numerous case reports and small open label uncontrolled trials. Rituximab was effective in inducing remission in some patients with severe LN, including some who were refractory to conventional immunosuppression.[13–15] In light of these promising results, the role of rituximab for LN is currently being investigated in a multicenter prospective placebo-controlled trial (LUNAR by Genetech) in which both arms of the study will receive MMF induction, but only one arm with receive concomitant rituximab.

Another novel targeted approach to the treatment of systemic lupus erythematosus involves blocking the interaction between T and B cells. The interaction of B7 molecules on B cells and CD28 on T cells provides an important co-stimulatory signal for T-cell activation and production of antibodies by B cells. Cytotoxic T lymphocyte antigen 4, a molecule structurally similar to CD28 on T cells has an

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inhibitory effect on T-cell activation by preventing B7/CD28 interaction. Cytotoxic T lymphocyte antigen 4-Ig, a fusion molecule that combines cytotoxic T lymphocyte antigen 4 and an immunoglobulin chain, was developed to take advantage of this regulatory potential. In murine models of lupus, cytotoxic T lymphocyte antigen 4-Ig has been shown to reduce autoantibody production, attenuate glomerulonephritis and prolong survival. Although this approach has not yet been applied in human LN, clinical trials using these monoclonal antibodies are being formulated. Earlier clinical trials using other monoclonal antibodies (IDEC-131, BG9588) targeting another important receptor ligand pair, CD40/CD40L, have proven unsuccessful, in part due to lack of efficacy and in part due to thrombotic complications.[16–18]

IgA NEPHROPATHY

There are only a few well-designed randomized controlled trials evaluating different therapies in IgA nephropathy (IgAN). The evidence for some 'nonspecific therapies' such as optimizing blood pressure control, blockade of the rennin–angiotensin–aldosterone system seems clear. There is even controlled data supporting the use of combined blockade of the rennin–angiotensin system using ACE inhibitors and AII receptor antagonists concomitantly. The benefit of the remainder of therapies including fish oils, tonsillectomy, and immunosuppression for IgAN remains controversial.

A number of studies have evaluated the role of fish oil in IgAN, but have led to conflicting results. A RCT by Pettersson *et al.* in Sweden found that fish oils provided no renoprotective effects and actually led to a greater decline in GFR than placebo (corn oil).[19] In contrast, the RCT of 106 IgAN patients from the Mayo Clinic clearly showed a benefit of fish oils.[20] Fewer patients treated with fish oil compared to placebo had a 50% increase in plasma creatinine (6 vs 33%, respectively). The fish oil arm also showed a slower decline in GFR, less need for dialysis and transplantation, and fewer deaths.[21] However, a more recent RCT by Hogg *et al.* showed no benefit following 2 years of treatment with fish oil compared to corticosteroids or placebo (abstract; Hogg R *et al.*, *J Am Soc Nephrol* 2003; **14**: 751A). At present, given the contradictory results, it is reasonable to allow patients decide for themselves whether the side effects and cost of fish oil therapy outweigh potentially unproven renal or cardiovascular benefits.

The role of immunosuppressive therapy in IgAN remains unclear. A number of studies have examined the role of corticosteroids in patients with IgAN and led to mixed results. In one controlled trial from Japan, Katafuchi *et al.*[22] randomized 90 IgAN patients with baseline serum creatinine (Scr) <1.5 mg/dl to receive oral prednisone (induction with 20 mg, gradually tapered to 5 mg/day over 2 years) or placebo. Blood pressure control was tight, although rennin–angiotensin–aldosterone system blockade was not used. Proteinuria declined more in the steroid-treated group, but renal survival was identical (85%) at follow up (65 ± 25 months) in both groups. In contrast, in a large well-designed RCT from Italy by Pozzi *et al.*[23] 86 IgAN patients (baseline Scr <1.5 mg/dl and 1–3.5 g/day proteinuria) were randomized to placebo or corticosteroids (pulse methylprednisolone at the beginning of each month followed by alternate day corticosteroids for 6 months). After 10 years of follow up, renal survival was better in the steroid group compared to placebo (97 vs 53%, respectively).[24] The steroid regimen led to few side effects. It is unknown whether replacing the corticosteroid pulses with high dose oral corticosteroids would have similar efficacy. It is also unknown whether tighter blood pressure control and universal use of rennin–angiotensin–aldosterone system blockade in the subjects would have minimized any of the observed benefits of steroids. Nevertheless, this study suggests a role for a 6-month trial of corticosteroids in IgAN patients with proteinuria of >1 g/day, yet preserved renal function. A recent meta-analysis also supports the use of corticosteroids in reducing proteinuria and preventing progression to end-stage renal disease, although this study was not controlled for blood pressure or use of angiotensin-converting enzyme inhibitors.

The role of CYC was studied by Ballardie and Roberts.[25] in 38 patients at high risk of progression (i.e., abnormal serum creatinine, rising by at least 15% in the year before entry and end-stage renal disease predicted within 5 years). Patients were randomized to either placebo or oral CYC (1.5 mg/kg/day for 3 months) plus steroids followed by AZA (1.5 mg/kg/day) for 2 or more years. The 5-year renal survival in the treated group was 72 vs 6% in the control group. This study may be faulted for suboptimal blood pressure control, insufficient use of rennin–angiotensin–aldosterone system blockade, small number of patients, and unusually poor survival rate of the placebo group. Clearly, more supportive evidence is required before this treatment can be widely accepted but it may be reasonable to use this therapy in a subset of patients with significant proteinuria and deteriorating renal function. Other investigators have suggested a clear beneficial role of CYC in IgAN patients with true crescentic glomerulonephritis when compared to the course of historical controls.

The role of MMF for IgAN has been evaluated in four major trials with conflicting results. Chen X *et al.*[26] and Chen Z *et al.*[27] compared MMF vs prednisone in 62 Chinese patients with IgAN (mean urine protein >2 g/day) and showed a benefit with respect to proteinuria in the MMF treatment arm. Serum creatinine was higher in the prednisone group at 72 weeks. Tang *et al.*[28] also showed a greater reduction in proteinuria in patients treated with MMF compared to placebo, but there was no difference in serum creatinine. In contrast, Maes *et al.*[29] randomized 34 IgAN patients (mean serum creatinine 1.4 mg/dl, urine protein excretion 1.6 g/day) to either MMF (2 g daily) or placebo and found no significant differences in creatinine or proteinuria at 3 years follow-up. Similarly, we were unable to demonstrate benefit of MMF in IgAN patients with moderately

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advanced disease in our single center-blinded RCT of MMF vs placebo.[30] Patients had a mean SCr of 2.4 mg/dl and mean urine protein excretion of 2.7 g/day and thus were at high risk of progressive renal disease. All patients had optimal BP control with angiotensin-converting enzyme inhibitors. This trial was prematurely terminated after enrolling only 40 patients due to the high rate of progression in both arms of the study. Thus, despite demonstrated benefit of MMF in other glomerular diseases, at present, there is insufficient evidence to support the use of MMF for the treatment of IgAN.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Focal segmental glomerulosclerosis (FSGS) is the most common cause of the idiopathic nephrotic syndrome (NS) in adult African-Americans. It is also the most common primary glomerular disease leading to end-stage renal disease in the United States. Although there have been few controlled, randomized trials of immunosuppressive regimens in treating adults with FSGS, both a prolonged course of corticosteroids and cyclosporine (CSA) are considered effective in inducing remissions of the NS and in preventing progressive renal failure. Cyclosporine has been shown to be more effective than placebo in an RCT in adult FSGS in which all patients were steroid resistant and 40% had failed cytotoxic therapy.[31] In the CSA arm, 70% of patients achieved a complete or partial remission of the NS vs only 4% of the placebo group. In addition, at 4 years follow up, fewer patients treated with CSA had a 50% decrease in GFR compared to placebo (25 vs 52%, respectively). Thus, CSA has proven to be an effective treatment for adults with FSGS. There is far less data on MMF in the treatment of FSGS.

One study by Choi *et al.*[32] examined the use of MMF in 18 FSGS patients (including 12 with renal insufficiency and nine with the NS), who were either steroid resistant or steroid/cyclosporine dependent. Following treatment with MMF and for many, concurrent steroids, the urine protein to creatinine ratio decreased to 48%, two patients achieved complete remission and six had partial remission of the NS. A second study examined the course of 18 steroid-resistant FSGS patients after MMF therapy.[33] All patients had the NS (mean proteinuria 9.1 g/day) and 75% of patients had also failed therapy with a cytotoxic agent and/or a calcineurin inhibitor previously. After 6 months of MMF, although there were no complete remissions, six had partial remissions, two had a 50% reduction in proteinuria, and two had a decrease in proteinuria below the nephrotic range. There were no changes in serum creatinine over the study period, and there was no significant toxicity from MMF. Currently there is a multicenter, RCT sponsored by the National Institutes of Health, comparing MMF to CSA in patients aged 3–40 years with steroid-resistant FSGS.

Sirolimus is widely used in renal transplantation. It was hoped that it would provide the efficacy of calcineurin inhibitors without significant nephrotoxicity. Data on its use in FSGS are conflicting. Two small uncontrolled, non-randomized trials suggest episodes of acute renal failure with this agent in FSGS patients (abstract: Cho M *et al.*, *J Am Soc Nephol* 2005; **16**: 775A). A third trial of 21 patients found major reductions in proteinuria and correction of sieving coefficient defects in a group of FSGS patients treated with sirolimus. (Tumlin *et al.* CJASN 2006) At present, with concerns of proteinuria in transplant patients treated with this drug, more data will be needed before it can be recommended for new trials treating FSGS in native kidneys.

MEMBRANOUS NEPHROPATHY

Membranous nephropathy (MN) remains the most common pattern of idiopathic NS in Caucasian adults.[34] It appears that only certain patients will benefit from immunosuppressive treatment, whereas others will do well over the long term with only 'nonspecific therapy'.[35] In general, patients with heavy persistent nephrotic range proteinuria, older female and male subjects, and those with renal dysfunction appear to benefit from the treatment.[36–39] A host of therapies have been recommended, but only a few have been investigated in RCTs. Cytotoxic agents such as chlorambucil and cyclosphosphamide have been shown to be effective in reducing proteinuria in patients with MN (Ponticelli C, Zucchelli P, Passerini P *et al.* A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int* 1995; **48**: 1600–1604; Ponticelli C, Altieri P, Scolari F *et al.* A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophosphamide in idiopathic membranous nephropathy. *J Am Soc Nephrol* 1998; **9**: 444–450; du Buf-Vereijken PW, Branten AJ, Wetallzelthis JF: Membranous Nephropathy Study Group. Cytotoxic therapy for membranous nephropathy and renal insufficiency: improved renal survival but high relapse rate. *Nephrol Dial Transplant* 2004; **19**: 1142–1148).

Cyclosporine has been shown to induce more complete and partial remissions of the NS in a randomized trial in which 51 patients were randomized to either CSA plus low-dose steroids vs steroids alone.[40] At the end of 6 months of treatment, 21 of 28 vs 5 of 23 placebo-treated patients were in partial or complete remission. The remission rate remained significant in favor of CSA at longer follow up, although few patients from either group progressed to renal failure.

MMF has been used in a series of uncontrolled trials in high-risk nephrotic patients with MN.[32,41] In 17 MN, patients treated with MMF, Choi *et al.*[32] reported a decrease in median urine protein/creatinine ratio from 7.3 (0.1, 18.5) to 1.5 ($<$0.1, 14.3) ($P = 0.001$), without a significant change in serum creatinine. In a study by Miller *et al.*,[41] 16 MN patients who had failed therapy with other immunosuppressive agents were treated for a mean duration of 8 months with MMF. Six patients had a $\geqslant$50% reduction in proteinuria.

Eculizumab is a humanized monoclonal antibody that prevents the activation of complement component C5. In a randomized, placebo-controlled trial in 200 patients with

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MN, eculizumab although well-tolerated, failed to show any significant reduction of proteinuria. There were concerns that the dosing schedules were inadequate as inhibition of complement was not uniformly demonstrated. (Appel G, Nachman P, Hogan S *et al.* Eculizumab (C5 complement inhibitor) in the treatment of idiopathic membranous nephropathy [Abstract]. *J Am Soc Nephrol* 2002; **13**: 668A–1857).

Rituximab was recently shown to reduce proteinuria in small uncontrolled trials of patients with MN.[42,43] In eight nephrotic patients with MN, Ruggenenti *et al.* studied the effects of treatment with Rituximab. CD20 cells remained depleted for up to 12 months. Five of the eight patients had a slow but progressive decline in proteinuria to subnephrotic levels over the first 6 months, and the three other patients had a smaller decline in proteinuria. This effect on proteinuria remained for up to 1 year after treatment. Adverse effects were rare. In a later analysis by the same group, rituximab was found to be effective only in those patients without interstitial fibrosis and/or significant renal dysfunction at the time of biopsy (abstract: Ruggenenti P *et al.*, *J Am Soc Nephrol* 2003; **14**: 528A).

It is clear that there is a need for newer, effective yet safe, immunosuppressive agents to treat a variety of glomerular diseases. Some new therapies have been adopted from use in transplantation, whereas others are derived from experiences in the oncology or rheumatologic arenas. Fortunately, many are being studied in a randomized, controlled fashion. These trials should lead to more specifically targeted treatment strategies for patients with glomerular diseases in the near future.

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EAST Search History

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(71) Applicants *(for all designated States except US)*: **ALEXION PHARMACEUTICALS, INC.** [US/US]; 352 Knotter Drive, Cheshire, CT 06410 (US). **THE UNIVERSITY OF WESTERN ONTARIO** [CA/CA]; 1151 Richmond Street North, Stevenson-Lawson Building, Room 328, London, Ontario N6A 5B8 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROTHER, Russell, P.** [US/US]; 25 Blue Trail Drive, Prospect, CT 06712 (US). **WANG, Hao** [CA/CA]; 480 Sandybrook Drive, London, Ontario N5X 4H2 (CA). **ZHONG, Zhen** [CA/CA]; 60 Nathaniel Court, London, Ontario N5X 2N5 (CA).

(74) Agents: **WU, Xuqiong** et al.; Fish & Neave IP Group of, Ropes & Gray LLP, One International Place, Boston, MA 02110-2624 (US).

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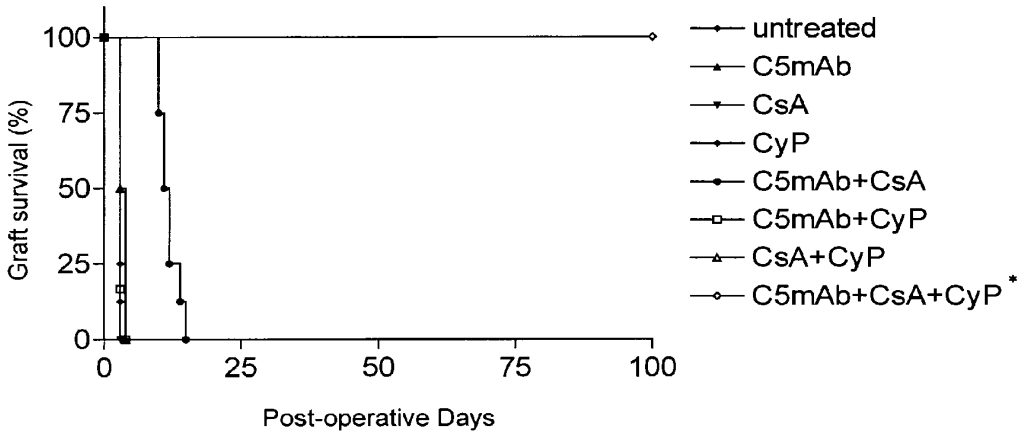

(57) Abstract: Methods of prolonging survival of allotransplanted cells, tissues or organs are presented. These methods are directed to administering to the allotransplant recipient an inhibitor of complement activity together with one or more immunosuppressants. The inhibitor of complement activity is administered chronically. These methods have been determined to aid in preventing chronic rejection of allografts. These methods can additionally be used in cases in which the recipient has been presensitized to the allograft or in which there is an ABO mismatch between the allograft and the recipient.

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PROLONGATION OF SURVIVAL OF AN ALLOGRAFT BY INHIBITING COMPLEMENT ACTIVITY

RELATED APPLICATIONS

This application claims the benefit of and priority to U.S. Provisional Patent Application No. 60/571,444, filed May 14, 2004, the disclosure of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The present disclosure relates to methods for prolonging survival of an allograft in a mammal. In particular, the present disclosure relates to prolonging survival of an allograft by administering an inhibitor of complement or terminal complement formation, especially an inhibitor of complement C5 cleavage, in addition to one or more drugs that are immunosuppressant.

BACKGROUND

Organ transplantation is the preferred treatment for most patients with chronic organ failure. Although transplantation of kidney, liver, lung, and heart offers an excellent opportunity for rehabilitation as recipients return to a more normal lifestyle, it is limited by the medical/surgical suitability of potential recipients, an increasing shortage of donors, and premature failure of transplanted organ function.

Transplantation of cells, tissues and organs has become very common and is often a life-saving procedure. Organ transplantation is the preferred treatment for most patients with chronic organ failure. Despite great improvement in treatments to inhibit rejection, rejection continues to be the single largest impediment to successful organ transplantation. Rejection includes not only acute rejection but also chronic rejection. One-year survival rates for transplanted kidneys average 88.3% with kidneys from deceased donors and 94.4% with kidneys received from living donors. The corresponding five year survival rates for the transplanted kidneys are 63.3% and 76.5% (OPTN/SRTR Annual Report, 2002). For livers the one year survival rates are 80.2% and 76.5% for livers from deceased and living donors, respectively. The corresponding five year liver graft survival rates are 63.5% and 73.0% (OPTN/SRTR Annual Report, 2002). The use of immunosuppressant drugs, especially cyclosporin A and more recently tacrolimus, has dramatically improved the success rate of organ transplantation especially by preventing acute rejection. But as the numbers above

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show, there is still a need to improve the success rates, both short-term and especially long-term. As seen from the above numbers for kidney and liver transplants, the five year failure rates for these transplanted organs are on the order of 25-35%. In the year 2001 alone there were more than 23,000 patients who received an organ transplant of which approximately 19,000 were kidney or liver (OPTN/SRTR Annual Report, 2002). For this one year of transplants alone, with present techniques it can be expected that approximately 5,000-6,000 of these transplanted kidneys and livers will fail within 5 years. These numbers do not even include other transplanted organs or transplanted tissues or cells such as bone marrow.

There are multiple types of transplants. These are described in Abbas et al., 2000. A graft transplanted from one individual to the same individual is called an autologous graft or autograft. A graft transplanted between two genetically identical or syngeneic individual is called a syngeneic graft. A graft transplanted between two genetically different individuals of the same species is called an allogeneic graft or allograft. A graft transplanted between individuals of different species is called a xenogeneic graft or xenograft. The molecules that are recognized as foreign on allografts are called alloantigens and those on xenografts are called xenoantigens. The lymphocytes or antibodies that react with alloantigens or xenoantigens are described as being alloreactive or xenoreactive, respectively.

Currently more than 40,000 kidney, heart, lung, liver and pancreas transplants are performed in the United States each year (Abbas et al., 2000). Other possible transplants include, but are not limited to, vascular tissue, eye, cornea, lens, skin, bone marrow, muscle, connective tissue, gastrointestinal tissue, nervous tissue, bone, stem cells, islets, cartilage, hepatocytes, and hematopoietic cells. Unfortunately, there are many more candidates for a transplant than there are donors. To overcome this shortage, a major effort is being made to learn how to use xenografts. While progress is being made in this field, the fact is that at present most transplants are allografts. An allogeneic transplant, while presently being more likely to be successful than a xenogeneic transplant, must surmount numerous obstacles to be successful. There are several types of immunological attacks made by the recipient against the donor organ which can lead to rejection of the allograft. These include hyperacute rejection, acute vascular rejection (including accelerated humoral rejection and de novo acute humoral rejection), and chronic rejection. Rejection is normally a result of T-cell mediated or humoral antibody attack, but may include additional secondary factors such as the effects of complement and cytokines.

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An ever growing gap between the number of patients requiring organ transplantation and the number of donor organs available has become a major problem throughout the world. Park et al., 2003. Individuals who have developed anti-HLA antibodies are said to be immunized or sensitized. Gloor, 2005. HLA sensitization is the major barrier to optimal utilization of organs from living donors in clinical transplantation (Warren et al., 2004) due to the development of severe antibody-mediated rejection (ABMR). For example, more than 50% of all individuals awaiting kidney transplantation are presensitized patients (Glotz et al., 2002) who have elevated levels of broadly reactive alloantibodies, resulting from multiple transfusions, prior failed allografts, or pregnancy (Kupiec-Weglinski, 1996). The role of ABMR is currently one of the most dynamic areas of study in transplantation, due to recognition that this type of rejection can lead to either acute or chronic loss of allograft function. Mehra et al., 2003. Numerous cases of ABMR, including hyperacute rejection (HAR) or accelerated humoral rejection (ACHR), have been reported that are characterized by acute allograft injury that is resistant to potent anti-T cell therapy, the detection of circulating donor specific antibodies, and the deposition of complement components in the graft. ABMR with elevated circulating alloantibodies and complement activation that occurs in 20-30% of acute rejection cases has a poorer prognosis than cellular rejection. Mauiyyedi et al., 2002.

Highly presensitized patients, who exhibit high levels of alloantibodies, usually suffer an immediate and aggressive HAR. In clinical practice, with great efforts and significant advances in technology, HAR is avoided by obtaining a pretransplant lymphocytotoxic cross-match to identify sensitized patients with antibodies specific for donor HLA antigens. However, circulating antibodies against donor HLA or other non-MHC endothelial antigens may also be responsible for a delayed form of acute humoral rejection, which is associated with an increased incidence of graft loss. Collins et al., 1999. Therefore, development of a novel presensitized animal model to mimic ABMR in clinical settings would be beneficial to studies on the mechanism, and to the much needed progress in the management of allograft rejection in presensitized hosts.

Some highly presensitized patients can benefit from intervention programs such as immunoadsorption (Palmer et al.,1989; Ross et al., 1993; Kriaa et al., 1995), plasmapheresis and intravenous immunoglobulin (Sonnenday et al., 2002; Rocha et al., 2003), that have been designed and implemented to temporarily eliminate anti-donor antibodies. However, in addition to their benefits, the aforementioned therapies carry with them numerous drawbacks

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as some individuals are less susceptible to their effects (Kriaa et al., 1995; Hakim et al., 1990; Glotz et al., 1993; Tyan et al., 1994); they are extremely expensive, time-consuming, and risky (Salama et al., 2001). Moreover, the transient and variable effect of these protocols has limited their impact. Glotz et al., 2002; Kupin et al., 1991; Schweitzer et al., 2000. Therefore, developing novel strategies to reduce the risk and cost in prevention of ABMR would be beneficial to presensitized recipients receiving an allograft.

SUMMARY

Accordingly, methods of prolonging survival of transplanted cells, tissues or organs are provided. In particular, methods of prolonging survival of allotransplanted cells, tissues or organs are provided. These methods are directed to using one or more immunosuppressants in addition to an inhibitor of complement activity. Use of one or more immunosuppressants and an inhibitor of complement activity in the manufacture of one or more medicaments or medicament packages is also provided. Such medicaments or medicament packages are useful in prolonging survival of an allograft in a subject mammal.

In certain embodiments, the inhibition of complement activity is effected by chronic administration of a drug directed against complement C5. A preferred drug that inhibits complement activity is an antibody specific to one or more components of complement, for example, C5. In certain preferred embodiments, the antibody inhibits the cleavage of C5 and thereby inhibits the formation of both C5a and C5b-9. The antibody may be, e.g., a monoclonal antibody, a chimeric antibody (e.g., a humanized antibody), an antibody fragment (e.g., Fab), a single chain antibody, an Fv, or a domain antibody. The recipient is also treated with one or more immunosuppressive drugs, for example, cyclosporin A.

In certain embodiments, either an MHC mismatched recipient (i.e., a mammalian recipient of an MHC mismatched allograft), a presensitized recipient or an ABO mismatched recipient (i.e., a mammalian recipient of an AMB mismatched allograft) is treated. In this model, the recipient is again chronically treated with a complement inhibitor, preferably an anti-C5 monoclonal antibody, together with immunosuppressive drugs, preferably a chronic administration of cyclosporin A and a short-term administration of cyclophosphamide. This triple therapy results in extended graft survival in the presensitized allotransplant recipient.

The present disclosure also provides methods of prolonging survival of an allograft in a mammalian recipient by administering to the recipient agents that modulate the level and/or ratio of subclasses and/or isotypes of anti-donor immunoglobulins (Ig) in the recipient. In

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certain embodiments, an agent that reduces the level of anti-donor IgG1 in the recipient is preferred. In certain embodiments, an agent that increases the level of anti-donor IgG2a and/or IgG2b in the recipient is preferred. In certain embodiments, an agent that reduces the ratio of anti-donor IgG1/anti-donor IgG2a or IgG2b in the recipient is preferred.

The present disclosure also provides a method of prolonging survival of an allograft in a second mammalian recipient using an allograft that has been accommodated in a first mammalian recipient (i.e., the allograft has prolonged survival in the first recipient). The present disclosure further provides an allograft that is resistant to anti-donor antibodies in a mammalian recipient, and the allograft is prepared from a first recipient that has accommodated the allograft. In preferred embodiments, the first recipient has accommodated the allograft by receiving a treatment as described herein, such a triple therapy treatment involving administering to the first recipient a drug that inhibits complement activity and two immunosuppressive agents.

Further provided are pharmaceutical packages. A pharmaceutical package of the present disclosure may comprise a drug that inhibits complement activity and at least one immunosuppressive agent. The pharmaceutical package may further comprise a label for chronic administration. The pharmaceutical package may also comprise a label for self-administration by a patient, for example, a recipient of a transplant graft, or instructions for a caretaker of a recipient of a transplant graft. In certain embodiments, the drug and the agent in the pharmaceutical package are in a formulation or separate formulations that are suitable for chronic administration and/or self-administration.

The present disclosure also provides lyophilized formulations and formulations suitable for injection. Certain embodiments provide a lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant. In preferred embodiments, the antibody formulation is suitable for chronic administration, for example, the antibody formulation is stable. Alternative embodiments provide an injection system comprising a syringe; the syringe comprises a cartridge containing an antibody that inhibits complement activity and is in a formulation suitable for injection.

An antibody employed in various embodiments of the present disclosure preferably inhibits the formation of terminal complement or C5a. In certain embodiments, antibody inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment. The whole antibody or antibody fragment may be a human, humanized,

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chimerized or deimmunized antibody or antibody fragment. In certain embodiments, the whole antibody or antibody fragment may inhibit cleavage of complement C5. In certain embodiments, the antibody fragment is a Fab, an F(ab')2, an Fv, a domain antibody, or a single-chain antibody. In preferred embodiments, the antibody fragment is pexelizumab. In alternative preferred embodiments, the whole antibody is eculizumab.

In certain embodiments, a drug, such as an antibody, that inhibits complement activity is present in unit dosage form, which can be particularly suitable for self-administration. Similarly, an immunosuppressive agent of the present disclosure may also be present in unit dosage form.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D show anti-donor antibody levels in presensitized versus unsensitized recipients under different treatments.

Figures 2A and 2B show comparison between triple therapy using anti-C5 antibody, CsA and CyP in presensitized allograft recipients and combination therapy using only anti-C5 antibody and CsA in presensitized allograft recipients. Figure 2A compares heart-allograft survival in various recipients under different treatments as indicated. Figure 2B shows histology and immunohistology, for example, for lymphocyte infiltration in heart allografts of recipients in different groups.

Figure 3 shows blocked terminal complement activity by anti-C5 antibody as compared to immunosuppressive agents.

Figures 4A-4D compare levels of anti-donor antibodies in presensitized recipients of allografts under monotherapy with anti-C5 antibody alone, double combination therapy with anti-C5 antibody and CsA, and triple combination therapy with anti-C5 antibody, CsA and CyP.

Figures 5A and 5B show change of ratios of IgG isotypes in allograft recipients that were untreated or under different treatments.

Figure 6 shows high-level expression of Bcl-2 and Bcl-xl proteins in long-term surviving heart grafts as compared to heart grafts of untreated animals.

Figure 7 shows improved second transplantation (re-transplantation) of an accommodated graft from a first transplantation recipient.

Figure 8 shows results from re-transplantation experiments.

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Figure 9 shows results from re-transplantation experiments.

DETAILED DESCRIPTION

Overview: Rejection of Transplants or Grafts

Hyperacute rejection occurs within minutes to hours after transplant and is due to preformed antibodies to the transplanted tissue antigens. It is characterized by hemorrhage and thrombotic occlusion of the graft vasculature. The binding of antibody to endothelium activates complement, and antibody and complement induce a number of changes in the graft endothelium that promote intravascular thrombosis and lead to vascular occlusion, the result being that the grafted organ suffers irreversible ischemic damage (Abbas et al., 2000). Hyperacute rejection is often mediated by preexisting IgM alloantibodies, e.g., those directed against the ABO blood group antigens expressed on red blood cells. This type of rejection, mediated by natural antibodies, is the main reason for rejection of xenotransplants. Hyperacute rejection due to natural IgM antibodies is no longer a major problem with allografts because allografts are usually selected to match the donor and recipient ABO type. Hyperacute rejection of an ABO matched allograft may still occur, usually mediated by IgG antibodies directed against protein alloantigens, such as foreign MHC molecules, or against less well defined alloantigens expressed on vascular endothelial cells. Such antibodies may arise as a result of prior exposure to alloantigens through blood transfusion, prior transplantation, or multiple pregnancies (this prior exposure being referred to as "presensitization"). Abbas et al., 2000.

Acute rejection is a process of vascular and parenchymal injury mediated by T cells, macrophages, and antibodies that usually begins after the first week of transplantation. Abbas et al., 2001. T lymphocytes play a central role in acute rejection by responding to alloantigens, including MHC molecules, present on vascular endothelial and parenchymal cells. The activated T cells cause direct lysis of graft cells or produce cytokines that recruit and activate inflammatory cells, which cause necrosis. Both $CD4^+$ and $CD8^+$ cells may contribute to acute rejection. The destruction of allogeneic cells in a graft is highly specific and a hallmark of $CD8^+$ cytotoxic T lymphocyte killing. Abbas et al., 2000. $CD4^+$ T cells may be important in mediating acute graft rejection by secreting cytokines and inducing delayed-type hypersensitivity-like reactions in grafts, with some evidence available that indicates that $CD4^+$ T cells are sufficient to mediate acute rejection. Abbas et al., 2000. Antibodies can also mediate acute rejection after a graft recipient mounts a humoral immune

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response to vessel wall antigens and the antibodies that are produced bind to the vessel wall and activate complement. Abbas et al., 2000.

Chronic rejection is characterized by fibrosis with loss of normal organ structures occurring over a prolonged period. The pathogenesis of chronic rejection is less well understood than that of acute rejection. Graft arterial occlusion may occur as a result of the proliferation of intimal smooth muscle cells (Abbas et al., 2000). This process is called accelerated or graft arteriosclerosis and can develop in any vascularized organ transplant within 6 months to a year after transplantation.

For a transplant to be successful, the several modes of rejection must be overcome. Multiple approaches are utilized in preventing rejection. This may require administration of immunosuppressants, often several types to prevent the various modes of attack, e.g., inhibition of T-cell attack, antibodies, and cytokine and complement effects. Prescreening of donors to match them with recipients is also a major factor in preventing rejection, especially in preventing hyperacute rejection. Immunoadsorption of anti-HLA antibodies prior to grafting may reduce hyperacute rejection. Prior to transplantation the recipient or host may be administered anti-T cell reagents, e.g., the monoclonal antibody OKT3, Anti-Thymocyte Globulin (ATG), cyclosporin A, or tacrolimus (FK 506). Additionally, glucocorticoids and/or azathioprine may be administered to the host prior to transplant. Drugs used to aid in preventing transplant rejection include, but are not limited to, ATG or ALG, OKT3, daclizumab, basiliximab, corticosteroids, 15-deoxyspergualin, cyclosporins, tacrolimus, azathioprine, methotrexate, mycophenolate mofetil, 6-mercaptopurine, bredinin, brequinar, leflunamide, cyclophosphamide, sirolimus, anti-CD4 monoclonal antibodies, CTLA4-Ig, anti-CD154 monoclonal antibodies, anti-LFA1 monoclonal antibodies, anti-LFA-3 monoclonal antibodies, anti-CD2 monoclonal antibodies, and anti-CD45.

Allografts are rejected in part by the activation of T cells. The transplant recipient mounts a rejection response following $CD4^+$ T cell recognition of foreign antigens in the allograft. These antigens are encoded by the major histocompatibility complex (MHC). There are both Class I and Class II MHC molecules. In humans the class I MHC molecules are HLA-A, HLA-B, and HLA-C. The class II MHC molecules in humans are called HLA-DR, HLA-DQ and HLA-DP. In mice the class I MHC molecules are H-2K, H-2D and H-2L and the class II MHC molecules are I-A and I-E. When $CD4^+$ T cells bind the foreign MHC antigens they are activated and undergo clonal proliferation. The activated T cells secrete cytokines which aid in activating monocytes/macrophages, B cells and cytotoxic $CD8^+$ T

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cells. The activated monocytes/macrophages release agents which result in tissue damage, the B cells produce alloantibodies which lead to complement mediated tissue destruction, and the $CD8^+$ T cells kill graft cells in an antigen-specific manner through induction of apoptosis and cell lysis.

Immunosuppressive Agents

The numerous drugs utilized to delay graft rejection (i.e., to prolong their survival) work in a variety of ways. Immunosuppressive agents are widely used. See Stepkowski, 2000, for a review of the mechanism of action of several immunosuppressive drugs. Cyclosporin A is one of the most widely used immunosuppressive drugs for inhibiting graft rejection. It is an inhibitor of interleukin-2 or IL-2 (it prevents mRNA transcription of interleukin-2). More directly, cyclosporin inhibits calcineurin activation that normally occurs upon T cell receptor stimulation. Calcineurin dephosphorylates NFAT (nuclear factor of activated T cells) enabling it to enter the nucleus and bind to interleukin-2 promoter. By blocking this process, cyclosporin A inhibits the activation of the $CD4^+$ T cells and the resulting cascade of events which would otherwise occur. Tacrolimus is another immunosuppressant that acts by inhibiting the production of interleukin-2.

Rapamycin (Sirolimus), SDZ RAD, and interleukin-2 receptor blockers are drugs that inhibit the action of interleukin-2 and therefore prevent the cascade of events described above.

Inhibitors of purine or pyrimidine biosynthesis are also used to inhibit graft rejection. These prevent DNA synthesis and thereby inhibit cell division including the ability of T cells to divide. The result is the inhibition of T cell activity by preventing the formation of new T cells. Inhibitors of purine synthesis include azathioprine, methotrexate, mycophenolate mofetil (MMF) and mizoribine (bredinin). Inhibitors of pyrimidine synthesis include brequinar sodium and leflunomide. Cyclophosphamide is an inhibitor of both purine and pyrimidine synthesis.

Yet another method for inhibiting T cell activation is to treat the recipient with antibodies to T cells. OKT3 is a murine monoclonal antibody against CD3 which is part of the T cell receptor. This antibody inhibits the T cell receptor and suppresses T cell activation.

Numerous other drugs and methods for delaying allotransplant rejection are known to and used by those of skill in the art. One approach has been to deplete T cells, e.g., by irradiation. This has often been used in bone marrow transplants, especially if there is a

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partial mismatch of major HLA. Administration to the recipient of an inhibitor (blocker) of the CD40 ligand-CD40 interaction and/or a blocker of the CD28-B7 interaction has been used (U.S. Patent 6,280,957). Published PCT patent application WO 01/37860 teaches the administration of an anti-CD3 monoclonal antibody and IL-5 to inhibit the Th1 immune response. Published PCT patent application WO 00/27421 teaches a method for prophylaxis or treatment of corneal transplant rejection by administering a tumor necrosis factor-α antagonist. Glotz et al. (2002) show that administration of intravenous immunoglobulins (IVIg) can induce a profound and sustained decrease in the titers of anti-HLA antibodies thereby allowing a transplant of an HLA-mismatched organ. Similar protocols have included plasma exchanges (Taube et al., 1984) or immunoadsorption techniques coupled to immunosuppressive agents (Hiesse et al., 1992) or a combination of these (Montgomery et al., 2000). Changelian et al. (2003) teach a model in which immunosuppression is caused by an oral inhibitor of Janus kinase 3 (JAK3) which is an enzyme necessary for the proper signaling of cytokine receptors which use the common gamma chain (γc) (Interleukins-2, -4, -7, -9, -15, -21), the result being an inhibition of T cell activation. Antisense nucleic acids against ICAM-1 have been used alone or in combination with a monoclonal antibody specific for leukocyte-function associated antigen 1 (LFA-1) in a study of heart allograft transplantation (Stepkowski, 2000). Similarly, an anti-ICAM-1 antibody has been used in combination with anti-LFA-1 antibody to treat heart allografts (Stepkowski, 2000). Antisense oligonucleotides have additionally been used in conjunction with cyclosporin in rat heart or kidney allograft models, resulting in a synergistic effect to prolong the survival of the grafts (Stepkowski, 2000). Chronic transplant rejection has been treated by administering an antagonist of TGF-β which is a cytokine involved in differentiation, proliferation and apoptosis (U.S. Patent Application Publication US 2003/0180301).

Complement and Transplant/Graft Rejection

The role of complement in transplant rejection is well known. This is especially true in the case of xenotransplantation, but complement also plays a role in allotransplant rejection. For review, see Platt and Saadi, 1999. One aspect of complement's role is that ischemia-reperfusion injury may occur at the time that an organ graft is reperfused with the blood of the recipient. Complement may also cause some manifestations of allograft rejection.

The complement system is described in detail in U.S. Patent 6,355,245. The complement system acts in conjunction with other immunological systems of the body to

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defend against intrusion of cellular and viral pathogens. There are at least 25 complement proteins, which are found as a complex collection of plasma proteins and membrane cofactors. The plasma proteins make up about 10% of the globulins in vertebrate serum. Complement components achieve their immune defensive functions by interacting in a series of intricate but precise enzymatic cleavage and membrane binding events. The resulting complement cascade leads to the production of products with opsonic, immunoregulatory, and lytic functions.

The complement cascade progresses via the classical pathway or the alternative pathway. These pathways share many components and, while they differ in their initial steps, they converge and share the same "terminal complement" components (C5 through C9) responsible for the activation and destruction of target cells.

The classical complement pathway is typically initiated by antibody recognition of and binding to an antigenic site on a target cell. The alternative pathway is usually antibody independent and can be initiated by certain molecules on pathogen surfaces. Both pathways converge at the point where complement component C3 is cleaved by an active protease (which is different in each pathway) to yield C3a and C3b. Other pathways activating complement attack can act later in the sequence of events leading to various aspects of complement function.

C3a is an anaphylatoxin. C3b binds to bacterial and other cells, as well as to certain viruses and immune complexes, and tags them for removal from the circulation. C3b in this role is known as opsonin. The opsonic function of C3b is considered to be the most important anti-infective action of the complement system. Patients with genetic lesions that block C3b function are prone to infection by a broad variety of pathogenic organisms, while patients with lesions later in the complement cascade sequence, i.e., patients with lesions that block C5 functions, are found to be more prone only to Neisseria infection, and then only somewhat more prone (Fearon, 1983).

C3b also forms a complex with other components unique to each pathway to form classical or alternative C5 convertase, which cleaves C5 into C5a and C5b. C3 is thus regarded as the central protein in the complement reaction sequence since it is essential to both the alternative and classical pathways (Wurzner et al., 1991). This property of C3b is regulated by the serum protease Factor I, which acts on C3b to produce iC3b. While still functional as opsonin, iC3b cannot form an active C5 convertase.

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C5 is a 190 kDa beta globulin found in normal serum at approximately 75 μg/mL (0.4 μM). C5 is glycosylated, with about 1.5-3 percent of its mass attributed to carbohydrate. Mature C5 is a heterodimer of a 999 amino acid 115 kDa alpha chain that is disulfide linked to a 656 amino acid 75 kDa beta chain. C5 is synthesized as a single chain precursor protein product of a single copy gene (Haviland et al., 1991). The cDNA sequence of the transcript of this gene predicts a secreted pro-C5 precursor of 1659 amino acids along with an 18 amino acid leader sequence.

The pro-C5 precursor is cleaved after amino acid 655 and 659, to yield the beta chain as an amino terminal fragment (amino acid residues +1 to 655) and the alpha chain as a carboxyl terminal fragment (amino acid residues 660 to 1658), with four amino acids deleted between the two.

C5a is cleaved from the alpha chain of C5 by either alternative or classical C5 convertase as an amino terminal fragment comprising the first 74 amino acids of the alpha chain (i.e., amino acid residues 660-733). Approximately 20 percent of the 11 kDa mass of C5a is attributed to carbohydrate. The cleavage site for convertase action is at or immediately adjacent to amino acid residue 733. A compound that would bind at or adjacent to this cleavage site would have the potential to block access of the C5 convertase enzymes to the cleavage site and thereby act as a complement inhibitor.

C5 can also be activated by means other than C5 convertase activity. Limited trypsin digestion (Minta and Man, 1977; Wetsel and Kolb, 1982) and acid treatment (Yamamoto and Gewurz, 1978; Vogt et al., 1989) can also cleave C5 and produce active C5b.

C5a is another anaphylatoxin. C5b combines with C6, C7, and C8 to form the C5b-8 complex at the surface of the target cell. Upon binding of several C9 molecules, the membrane attack complex (MAC, C5b-9, terminal complement complex-TCC) is formed. When sufficient numbers of MACs insert into target cell membranes the openings they create (MAC pores) mediate rapid osmotic lysis of the target cells. Lower, non-lytic concentrations of MACs can produce other effects. In particular, membrane insertion of small numbers of the C5b-9 complexes into endothelial cells and platelets can cause deleterious cell activation. In some cases activation may precede cell lysis.

As mentioned above, C3a and C5a are anaphylatoxins. These activated complement components can trigger mast cell degranulation, which releases histamine and other mediators of inflammation, resulting in smooth muscle contraction, increased vascular

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permeability, leukocyte activation, and other inflammatory phenomena including cellular proliferation resulting in hypercellularity. C5a also functions as a chemotactic peptide that serves to attract pro-inflammatory granulocytes to the site of complement activation.

Complement-binding recipient antibodies to donor alloantigens are considered to be the main cause of hyperacute graft rejection. Owing to pretransplant crossmatch testing, this prototype of humoral rejection is now rarely observed (Regele et al., 2001). Data are now showing that humoral immune mechanisms might contribute to other types of allograft rejection (Regele et al., 2001). High levels of panel reactive antibodies indicating humoral presensitization were found to be associated with inferior kidney graft survival (Opelz, 1992), the appearance of alloantibodies during the post-transplant period has been reported to predict poor graft outcome (Jeannet et al., 1970; Halloran et al., 1992), and selective removal of recipient IgG by immunoadsorption reversed some rejection episodes indicating the contribution of humoral immune mechanisms to rejection (Persson et al., 1995; Böhmig et al., 2000). Complement activation within a graft might indicate antibody-mediated graft injury. The complement cleavage product C4d is a marker for activation of the antibody-dependent classical pathway. Capillary C4d deposits in kidney allograft biopsies were associated with poor graft outcome.

Recently increasing evidence indicates that complement activation significantly contributes to the sensitization of allograft recipients and the development of tissue injury in allografts (Platt et al., 1999). Antibodies are the most thoroughly investigated mediators of activating the classical complement pathway. Clinically, alloantibodies are known to activate complement (Baldwin et al., 2001). Halloran and Collins indicate that C4d deposition in peritubular capillaries of renal allografts is a sensitive and diagnostic marker of acute humoral rejection that correlates strongly with the presence of circulating donor-specific antibodies (Collins et al., 1999; Halloran, 2003). Further supporting evidence is seen in animals with complement inhibition (Pratt et al., 1996; Pruitt et al., 1991; Forbes et al., 1978) or deficiency (Pratt et al., 2000; Baurer et al., 1995) which exhibit significantly reduced inflammatory injury and lowered anti-donor immune responses. In ABMR, complement is suggested to be activated by the classical pathway and to play a key role in the pathogenesis (Collard et al., 1997). Although the role of complement in HAR or acute vascular rejection (AVR) following xenotransplantation has been well documented (Platt et al., 1999), precise mechanisms of complement in the pathogenesis of ABMR following allotransplantation has not yet been elucidated.

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The C5 component of complement is cleaved to form products with multiple proinflammatory effects and thus represents an attractive target for complement inhibition within the immune-mediated inflammatory response. As described above, C5a is a powerful anaphylatoxin and chemotactic factor. Cellular activation by C5a induces the release of multiple additional inflammatory mediators (Jose et al., 1983). The complement activation pathways (classical, alternative, or mannan-binding lectin pathway) ultimately lead to the formation of the cytolytic membrane attack complex C5b-9 (Kirschfunk, 2001), which can mediate both direct tissue injury by cell lysis, and proinflammatory cell activation at sublytic doses (Saadi et al., 1995; Papadimitriou et al., 1991). Therefore, blocking both C5a and C5b-9 generation may be required for the optimal inhibition of complement-mediated inflammatory response following transplantation. At the same time, inhibition of the complement cascade at C5 does not impair the generation of C3b, preserving C3b-mediated opsonization of pathogenic microorganisms as well as solubilization and clearance of immune complexes (Liszewski, 1993).

The beneficial effect of anti-C5 mAb has previously been reported in several experimental models including myocardial reperfusion (Vakeva et al., 1998), systemic lupus erythematosus (Wang et al., 1996) and rheumatoid arthritis (Wang et al., 1995); as well as in human clinical trials (Kirschfink, 2001) of autoimmune disease, cardiopulmonary bypass and acute myocardial infarction. In addition, complement inactivation by a functionally blocking anti-C5 monoclonal Ab (mAb) prevented HAR in xenotransplantation models (Kroshus et al., 1995; Wang et al., 1999).

Methods of delaying allotransplant rejection by administration of complement inhibitors have been tested. Published PCT patent application WO 92/10205 discloses the use of a combination of cyclosporin and a soluble complement receptor (sCR1) to inhibit rejection of a cardiac allotransplant in a presensitized rat model. Complement receptor 1 binds complements C3b and C4b. Soluble forms of complement receptor 1 occur naturally or can be generated via recombinant DNA procedures. These soluble complement receptors have inhibited in vitro the consequences of complement activation (U.S. Patent 6,057,131). In WO 92/10205, rats, which had been presensitized to the cardiac allograft they were receiving, were administered cyclosporin A intramuscularly at 10 mg/kg/day beginning two days prior to transplant and continued until the time of graft rejection. Additionally, soluble complement receptor 1 (sCR1) was administered as a single intravenous bolus at 15 mg/kg immediately prior to reperfusion of the graft. Control animals with no drug treatment had the

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graft rejected at an average of 3.8 days. Those administered cyclosporin A alone rejected the grafts at an average of 57 days (this was quite variable with two rats rejecting quickly at 2 and 4 days and a third rat rejecting at 166 days). Rats administered sCR1 alone rejected the grafts at an average of 44 days. Those rats administered the combination of cyclosporin A and sCR1 rejected the grafts at an average of 147 days. The combination of chronic cyclosporin A and single bolus sCR1 was seen to result in a synergistic effect greatly prolonging the time until graft rejection. Earlier studies by Pruitt and Bollinger (1991) used a similar model of a presensitized rat allograft to show that administration of sCR1 alone to inactivate complement resulted in increased time before graft rejection.

Sims et al. (U.S. Patent 5,135,916) suggest using inhibitors of complement, e.g., CD59 or antibodies against C7 or C9 to block the formation of the C5b-9 complex, to treat the vascular endothelium of organs and tissues to be transplanted. This would prevent the C5b-9 initiated cell necrosis. The C5b-9 inactivators would be added to the perfusate or storage medium to protect the vascular lining cells from ongoing complement activation during in vitro storage. Additionally the organ or tissue would be protected from the cytolytic and thrombotic effects arising from complement activation initiated upon transplantation, thereby circumventing complement mediated acute rejection. Sims et al. (U.S. Patent 5,573,940 and U.S. Patent 6,100,443) also teach a method of expressing CD59 in the transplanted tissue or organ to protect the transplanted organ from rejection. This can be accomplished by transfecting the cells being transplanted.

Although the several drugs developed to date in combination with methods of prescreening donors and recipients to match the donor allograft to the recipient have over time increased the average length of time of survival of allografts, many allografts are nonetheless rejected during the life-time of the recipient. In general, the prior art advances have mainly been directed to overcoming acute graft rejection. Further, the role of activated terminal complement components in antibody-mediated allograft rejection has not been examined using inhibitors that specifically target the complement cascade at the C5 protein level. The methods described herein and as exemplified in the Examples advance the allotransplant art by inhibiting chronic rejection of allografts, in particular, allografts in a presensitized recipient. New methods are presented for further prolonging allograft survival by using a proper combination of immunosuppressive drugs in combination with a chronic administration of a complement inhibitor.

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Methods and Uses

The methods disclosed herein are used to prolong allograft survival. The methods generally include administering an inhibitor of complement activity in combination with one or more immunosuppressants.

Suitable complement inhibitors are known to those of skill in the art. Antibodies can be made to individual components of activated complement, e.g., antibodies to C5a, C7, C9, etc. (see, e.g., U.S. Patent 6,534,058; published U.S. patent application US 2003/0129187; and U.S. Patent 5,660,825). Proteins are known which inhibit complement-mediated lysis, including CD59, CD55, CD46 and other inhibitors of C8 and C9 (see, e.g., U.S. Patent 6,100,443). U.S. Patent 6,355,245 teaches an antibody which binds to C5 and prevents it from being cleaved into C5a and C5b thereby preventing the formation not only of C5a but also the C5b-9 complex. Proteins known as complement receptors and which bind complement are also known (see, Published PCT Patent Application WO 92/10205 and U.S. Patent 6,057,131). Use of soluble forms of complement receptors, e.g., soluble CR1, can inhibit the consequences of complement activation such as neutrophil oxidative burst, complement mediated hemolysis, and C3a and C5a production. Those of skill in the art recognize the above as some, but not all, of the known methods of inhibiting complement and its activation.

Suitable immunosuppressants include, but are not limited to, ATG or ALG, OKT3, daclizumab, basiliximab, corticosteroids, 15-deoxyspergualin, cyclosporins, tacrolimus, azathioprine, methotrexate, mycophenolate mofetil, 6-mercaptopurine, bredinin, brequinar, leflunamide, cyclophosphamide, sirolimus, anti-CD4 monoclonal antibodies, CTLA4-Ig, anti-CD154 monoclonal antibodies, anti-LFA1 monoclonal antibodies, anti-LFA-3 monoclonal antibodies, anti-CD2 monoclonal antibodies, and anti-CD45.

An allograft can include a transplanted organ, part of an organ, tissue or cell. These include, but are not limited to, heart, kidney, lung, pancreas, liver, vascular tissue, eye, cornea, lens, skin, bone marrow, muscle, connective tissue, gastrointestinal tissue, nervous tissue, bone, stem cells, islets, cartilage, hepatocytes, and hematopoietic cells.

At least part of the reason for the failure of allografts is that one response by the recipient of an allograft is the activation of complement. This results in the formation of C5a and C5b-9 which are potent proinflammatory molecules which aid in causing graft failure. Without wishing to be bound by any proposed theory, Applicants theorized that inhibiting the

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formation of C5a and C5b-9 or inhibiting C5a and C5b-9 which was present would aid in preventing graft failure. Furthermore, it was theorized that so long as the allograft is present, the recipient will continue to attempt to mount an immune response against the graft, and this response will include attempts to produce C5a and C5b-9. If not prevented, this complement response will lead to acute vascular rejection in the short term and could contribute to chronic graft rejection in the long term. Prior art methods of using inhibitors of complement activity were limited to administering these inhibitors only at the time of transplant. This was helpful in preventing acute rejection, but as the results disclosed herein illustrate, improved results are obtained by administration of such inhibitors for a longer term. This long-term administration aids in preventing a chronic rejection of the allograft as opposed to only aiding in preventing an acute rejection. The result is a longer term survival of the allograft as compared to either not administering an inhibitor of complement activity or administering such an inhibitor only at the time of transplant of the allograft. Although very commonly it is desirable that the allograft will survive for the remaining lifetime of the recipient, there are times when the allograft is needed only for a shorter length of time, e.g., a bridge organ to bridge the time until the recipient's own organ can recover on its own, at which time the allograft will no longer be needed. The length of time such a graft will be needed will vary, but will usually be longer than the time at which acute rejection would occur and may be long enough for chronic rejection to occur. This period of desired survival for a bridge graft may be several months, e.g., six months.

To prove that long-term inhibition of complement activity will prolong allograft survival, experiments were performed in which complement activation was inhibited in a chronic fashion and not merely at the time of transplant. Chronic treatment means treatment during an extended period up to the lifetime of the allograft. This can be daily treatment but is not limited to daily treatment. Chronic treatment will maintain an effective amount of the drug in the allograft recipient. For example, a preferred method is to include the anti-C5 monoclonal antibody eculizumab in the treatment. In studies of persons suffering from paroxysmal nocturnal hemoglobinuria (PNH), eculizumab has been administered at a dose of 900 mg/patient once every 12-14 days. This dosing has been found to completely and consistently block terminal complement activity and has greatly inhibited the symptoms of PNH (Hillmen et al., 2004). The administered dose is able to block the effects of complement for approximately two weeks before the eculizumab is inactivated or removed from the body. Therefore, a chronic treatment of eculizumab may be, e.g., the administration

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of 900 mg to the allograft recipient once every two weeks for the remaining life-time of the patient. Similarly, other drugs can be delivered chronically as needed, whether this is on a daily basis or another schedule is required to maintain an effective amount of the drug in the allograft recipient. Because it is well known that graft rejection can be caused by more than just complement activation, e.g., by T cell activity, the experiments included immunosuppressants such as cyclosporin to further aid in preventing graft rejection.

A preferred method of inhibiting complement activity is to use a monoclonal antibody which binds to complement C5 and prevents C5 from being cleaved. This prevents the formation of both C5a and C5b-9 while at the same time allowing the formation of C3a and C3b which are beneficial to the recipient. Such antibodies that are specific to human complement are known (U.S. Patent 6,355,245). These antibodies disclosed in U.S. Patent 6,355,245 include both a whole or full-length antibody (now named eculizumab) and a single-chain antibody (now named pexelizumab). A similar antibody against mouse C5 is called BB5.1 (Frei et al., 1987). BB5.1 was utilized in the experiments set forth below. Antibodies to inhibit complement activity need not be monoclonal antibodies. They can be, e.g., polyclonal antibodies. They may additionally be antibody fragments. An antibody fragment includes, but is not limited to, an Fab, F(ab'), $F(ab')_2$, a single-chain antibody, a domain antibody and an Fv. Furthermore, it is well known by those of skill in the art that antibodies can be humanized (Jones et al., 1986), chimerized, or deimmunized. An antibody may also comprise a mutated Fc portion, such that the mutant Fc does not activate complement. The antibodies to be used in the present disclosure may be any of these. It is preferable to use humanized antibodies when the recipient of the allograft is a human.

Administration and Formulations

Administration of the inhibitor of complement activity is performed according to methods known to those of skill in the art. These inhibitors are administered preferably before the time of allograft transplantation or at the time of transplantation with administration continuing in a chronic fashion. These inhibitors can additionally be administered during a rejection episode in the event such an episode does occur.

The present disclosure also provides uses of a drug that inhibits complement activity and an immunosuppressive agent in the manufacture of a medicament or medicament package. Such medicament or medicament package is useful in prolonging allograft survival in a recipient, in particular, chronic survival of the allograft. In preferred embodiments, the

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medicament or medicament package is formulated and prepared such that it is suitable for chronic administration to the recipient of the allograft, for example, stable formulations are employed. In certain embodiments, the medicament or medicament package is formulated and prepared such that it is suitable for concurrent administration of the drug that inhibits complement activity and the immunosuppressive drug to the recipient of the allograft. In certain embodiments, the medicament or medicament package is formulated and prepared such that it is suitable for sequential (in either order) administration of the drug that inhibits complement activity and the immunosuppressive drug to the recipient of the allograft.

A pharmaceutical package of the present disclosure may comprise a drug that inhibits complement activity and at least one immunosuppressive agent. The pharmaceutical package may further comprise a label for chronic administration. The pharmaceutical package may also comprise a label for self-administration by a patient, for example, a recipient of a transplant graft, or instructions for a caretaker of a recipient of a transplant graft. In certain embodiments, the drug and the agent in the pharmaceutical package are in a formulation or separate formulations that are suitable for chronic administration and/or self-administration.

The present disclosure also provides lyophilized formulations and formulations suitable for injection. Certain embodiments provide a lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant. In preferred embodiments, the antibody formulation is suitable for chronic administration, for example, the antibody formulation stable. Alternative embodiments provide an injection system comprising a syringe; the syringe comprises a cartridge containing an antibody that inhibits complement activity and is in a formulation suitable for injection.

An antibody employed in various embodiments of the present disclosure preferably inhibits the formation of terminal complement or C5a. In certain embodiments, antibody inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment. The whole antibody or antibody fragment may be a human, humanized, chimerized or deimmunized antibody or antibody fragment. In certain embodiments, the whole antibody or antibody fragment may inhibit cleavage of complement C5. In certain embodiments, the antibody fragment is a Fab, an F(ab')2, an Fv, a domain antibody, or a single-chain antibody. In preferred embodiments, the antibody fragment is pexelizumab. In alternative preferred embodiments, the whole antibody is eculizumab.

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In certain embodiments, a drug, such as an antibody, that inhibits complement activity is present in unit dosage form, which can be particularly suitable for self-administration. Similarly, an immunosuppressive agent of the present disclosure may also be present in unit dosage form. A formulated product of the present disclosure can be included within a container, typically, for example, a vial, cartridge, prefilled syringe or disposable pen. A doser such as the doser device described in U.S. Patent 6,302,855 may also be used, for example, with an injection system of the present disclosure.

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

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

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

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

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

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

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

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

Accordingly, a stable lyophilized antibody formulation can be prepared using a lyoprotectant (preferably a sugar such as sucrose or trehalose), which lyophilized formulation

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can be reconstituted to generate a stable reconstituted formulation having an antibody concentration which is significantly higher (e.g. from about 2-40 times higher, preferably 3-10 times higher and most preferably 3-6 times higher) than the antibody concentration in the pre-lyophilized formulation. Such high protein concentrations in the reconstituted formulation are considered to be particularly useful where the formulation is intended for subcutaneous administration. Despite the very high protein concentration in the reconstituted formulation, the reconstituted formulation can be stable (i.e. fails to display significant or unacceptable levels of chemical or physical instability of the protein) at 2-8 °C. for at least about 30 days. See U.S. Patent 6,821,515. In certain embodiments, the reconstituted formulation is isotonic.

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

The present disclosure also provides a method for preparing a formulation comprising the steps of: (a) lyophilizing a mixture of an antibody and a lyoprotectant; and (b) reconstituting the lyophilized mixture of step (a) in a diluent such that the reconstituted formulation is isotonic and stable.

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

An injection system of the present disclosure may employ a medication delivery pen as described in U.S. Patent 5,308,341. Medication delivery pens have been developed to facilitate the self-administration of medication. A medication of the present disclosure can be a drug that inhibits complement activity, for example an antibody specific to complement C5,

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and/or an immunosuppressive agent. One medication delivery pen includes a vial holder into which a vial of insulin or other medication may be received. The vial holder is an elongate generally tubular structure with proximal and distal ends. The distal end of the vial holder includes mounting means for engaging a double-ended needle cannula. The proximal end also includes mounting means for engaging a pen body which includes a driver and dose setting apparatus. A disposable medication containing vial for use with the prior art vial holder includes a distal end having a pierceable elastomeric septum that can be pierced by one end of a double-ended needle cannula. The proximal end of this vial includes a stopper slidably disposed in fluid tight engagement with the cylindrical wall of the vial. This medication delivery pen is used by inserting the vial of medication into the vial holder. A pen body then is connected to the proximal end of the vial holder. The pen body includes a dose setting apparatus for designating a dose of medication to be delivery by the pen and a driving apparatus for urging the stopper of the vial distally for a distance corresponding to the selected dose.

The user of the pen mounts a double-ended needle cannula to the distal end of the vial holder such that the proximal point of the needle cannula pierces the septum on the vial. The patient then selects a dose and operates the pen to urge the stopper distally to deliver the selected dose. The dose selecting apparatus returns to zero upon injection of the selected dose. The patient then removes and discards the needle cannula, and keeps the prior art medication delivery pen in a convenient location for the next required medication administration. The medication in the vial will become exhausted after several such administrations of medication. The patient then separates the vial holder from the pen body. The empty vial may then be removed and discarded. A new vial can be inserted into the vial holder, and the vial holder and pen body can be reassembled and used as explained above.

Accordingly, a medication delivery pen generally has a drive mechanism for accurate dosing and ease of use. A dosage mechanism such as a rotatable knob allows the user to accurately adjust the amount of medication that will be injected by the pen from a prepackaged vial of medication. To inject the dose of medication, the user inserts the needle under the skin and depresses the knob once as far as it will depress. The pen may be an entirely mechanical device or it may be combined with electronic circuitry to accurately set and/or indicate the dosage of medication that is injected into the user. See U.S. Patent 6,192,891.

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The present disclosure also presents controlled-release or extended-release formulations suitable for chronic and/or self-administration of a medication.

The various formulations can be administered to a patient in need of treatment (e.g., a recipient of an allograft) with the medication (e.g., an antibody of the present disclosure and at least one immunosuppressive agent) by intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

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

The present methods and uses are described with reference to the following Examples, which are offered by way of illustration and are not intended to limit the disclosure in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized. The following abbreviations are used herein: ABMR, antibody-mediated rejection; ACHR, accelerated humoral rejection; ACR, acute cellular rejection; AVR, acute vascular rejection; CsA, cyclosporin; CyP, cyclophosphamide; HAR, hyperacute rejection; MCP-1, monocyte chemotactic protein 1; MST, mean survival time; POD, postoperative day.

Example 1

Methods

Animals and Immunosuppressive Drugs Male adult C3H (H-2^k) mice and BALB/c (H-2^d) mice (Jackson Labs, Bar Harbor, Maine) weighing 25-30 g were chosen as donors and recipients, respectively. In the groups receiving immunosuppression, the recipients were injected with CsA (15 mg/kg/day, s.c., daily from day 0 to endpoint rejection or until day 100), or with CyP (40 mg/kg/day, i.v., on day 0 and 1), or with anti-C5 mAb (clone BB5.1, Alexion Pharmaceuticals Inc., 40 mg/kg/day, i.p., day 0–2, followed by twice a week, day 0-60). Animals were housed under conventional conditions at the Animal Care Facility,

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University of Western Ontario, and were cared for in accordance with the guidelines established by the Canadian Council on Animal Care. Olfert et al., 1993.

Skin Presensitization Full-thickness skin grafts taken from C3H donors were cut into square pieces of 1×1 cm^2 and transplanted onto the back of the BALB/c recipients' thorax one week prior to heart transplantation from the same donors. Rejection was defined as complete necrosis of the skin grafts.

Abdominal and Cervical Cardiac Transplantation Seven days after skin presensitization, C3H mouse hearts were transplanted into the abdomen of presensitized BALB/c recipients by anastomosing the donor aorta and recipient aorta, and the donor pulmonary artery and recipient inferior vena cava. In the groups with re-transplantation, second heart grafts harvested from either naive C3H mice or long-term surviving presensitized BALB/c recipients were transplanted into the cervical area of the recipients carrying a long-term surviving first abdominal heart graft by anastomosing the donor aorta and recipient carotid artery, and the donor pulmonary artery and recipient external jugular vein (end-to-side). The heart grafts were monitored daily until rejection unless otherwise indicated and rejection was defined as complete cessation of pulsation.

Experimental Groups Presensitized recipients were randomly assigned to eight groups, each consisting of eight animals: Group 1, mice with no treatment; Group 2, mice treated with CsA; Group 3, mice treated with CyP; Group 4, mice treated with CsA plus CyP; Group 5, mice treated with anti-C5 mAb; Group 6, mice treated with anti-C5 mAb plus CsA; Group 7, mice treated with anti-C5 mAb plus CyP; Group 8, mice treated with anti-C5 mAb in combination of CsA and CyP. When cardiac impulses were no longer palpable or at POD100, the grafts were removed for routine histology, immunohistochemistry and western blot analysis, serum samples were collected for flow cytometric analysis and complement hemolytic assay. Five additional animals were placed and sacrificed in groups 6 and 8 on POD3 (MST for groups 1-5, 7) to allow for comparisons at a uniform time point. Serum samples were also collected on POD 11, 21, 28 and 60 in Group 8 for detecting the sequential changes of anti-donor antibody levels and complement activity. In addition, when triple therapy treated presensitized recipients carried a first heart graft for 100 days, they were re-transplanted with a second heart. A naive C3H heart or a 100-day surviving C3H heart from another presensitized BALB/c recipient was used as the second heart. Eight animals were included in each re-transplant group.

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Graft Histology Tissue samples were fixed in 10% buffered formaldehyde. Specimens were then embedded in paraffin, and sectioned for H&E staining. The microscopic sections were examined in a blinded fashion for severity of rejection by a pathologist. Criteria for graft rejection included the presence of vasculitis, thrombosis, hemorrhage and lymphocyte infiltration. These changes were scored as: 0, no change; 1, minimum change; 2, mild change; 3, moderate change; or 4, marked change.

Immunohistochemistry Four micrometer sections were cut from tissue samples embedded in Tissue-Tek O.C.T gel (Optimum Cutting Temperature, Skura Finetek, Torrance, CA) mounted on gelatin-coated glass microscope slides and stained by a standard indirect avidin-biotin immunoperoxidase staining method using an Elite Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA). Specimens were stained for $CD4^+$ and $CD8^+$ cells with biotin-conjugated rat anti-mouse CD4 mAb (clone YTS 191.1.2, Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) and biotin-conjugated rat anti-mouse CD8 mAb (clone 53-6.7, Pharmingen, Franklin Lakes, NJ), respectively. Intragraft monocyte/macrophage infiltration was detected by staining with biotin-conjugated rat anti-mouse Mac-1 mAb (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada). Mouse IgG and IgM deposition in grafts was detected using biotin-conjugated goat anti-mouse-IgG and goat anti-mouse-IgM (Cedarlane). For identification of complement deposition, sections were serially incubated with goat anti-C3 or anti-C5 polyclonal Abs (Quidel, San Diego, CA), biotinylated rabbit anti-goat IgG (Vector Laboratories), and HRP-conjugated-streptavidin (Zymed Laboratories, South San Francisco, CA). Slides were washed with phosphate-buffered saline between steps, and examined under light microscopy. Negative controls were performed by omitting the primary antibodies. The immunostaining was scored in five high-power fields of each section, and five independent experiments were performed. The sections of immunoperoxidase staining were graded from 0 to 4+ according to the staining intensity: 0, negative; 1+, equivocal; 2+, weak staining; 3+, moderate staining; and 4+, very intensive staining.

Flow Cytometry The circulating anti-donor specific IgG and IgM antibodies were evaluated in the recipient serum by FACScan flow cytometry (Becton Dickinson, Mountain View, CA). Glotz et al., (1993); Tyan et al. (1994). Briefly, C3H mouse splenocytes were isolated and incubated at 37 °C for 30 minutes with serum from naive control and experimental groups. To stain for total IgG, IgG1, IgG2a, IgG2b and IgM, the cells were washed and incubated with FITC-conjugated goat antibody specific for the Fc portion of mouse IgG or with

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phycoerythrin-conjugated goat antibody specific for the μ-chain of mouse IgM (Jackson ImmunoResearch Laboratories, West Grove, PA), or with FITC-conjugated goat anti-mouse IgG1 (CALTAG Laboratories, Burlingame, CA), or with FITC-conjugated goat anti-mouse IgG2a (CALTAG), or with FITC-conjugated goat anti-mouse IgG2b (CALTAG). After 1 hour of staining at 4 °C, the cells were washed with PBS, resuspended at 5×10^6/mL, and analyzed by flow cytometry for mean channel fluorescence intensity, which represents the antibody-binding reactivity.

Complement Hemolytic Assay The purified anti-C5 mAb was serially diluted twofold (175-0.1 μg/ml) in GVB^{2+} buffer (gelatin Veronal-buffered saline: 0.1% gelatin, 141 mM NaCl, 0.5 mM $MgCl_2$, 0.15 mM $CaCl_2$, and 1.8 mM sodium barbital) and added in triplicate (50 μl/well) to a 96-well plate. BALB/c mouse serum was diluted to 40% v/v with GVB^{2+} buffer and added (50 μl/ml) to the rows of the same 96-well plate such that the final concentration of BALB/c mouse serum in each well was 20%. The plate was then incubated at room temperature for approximately 30 min while chicken erythrocytes were prepared. Chicken erythrocytes were washed 5×1 ml with GVB^{2+} buffer and resuspended to a final concentration of 5×10^7/ml in GVB^{2+}. Four milliliters of the chicken erythrocytes were sensitized by adding anti-chicken RBC polyclonal antibody (Intercell Technologies, Hopewell, NJ, 0.1% v/v) and the cells were incubated at 4 °C for 15 min with frequent vortexing. The cells were then washed 2×1 ml with GVB^{2+} and resuspended to a final volume of 2.4 ml in GVB^{2+}. The chicken erythrocytes (30 μl/well, 2.5×10^6 cells) were added to the plate containing serum and anti-C5 mAb as described above, mixed well, and incubated at 37 °C for 30 min. The plate was then centrifuged at 1000×g for 2 min, and 85 μl of the supernatant was transferred to a new 96-well microtiter plate. The plate was read at OD 415 nm using a microplate reader and the percentage of hemolysis was determined using this formula:

$$\%\ \text{hemolysis} = 100 \times \frac{(\text{OD sample}) - (\text{OD } GVB^{2+} \text{ control})}{(\text{OD 100\% lysed control}) - (\text{OD } GVB^{2+} \text{ control})}$$

with 100% lysed control obtained by the addition of 100 μl GVB^{2+} containing 0.1% NP-40 to the 30 μg/ml of chicken erythrocytes as prepared above.

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Western Blot Analysis Sonication of frozen heart samples was performed in RIPA lysis buffer (Santa Cruz Biotechnology, Inc.) at 4 °C for 1 minute at 10-second intervals, followed by microcentrifugation at 13,000 rpm for 10 minutes at 4 °C. Clarified supernatants were immediately quantitated in triplicate for protein content using Detergent-compatible protein assay kit (BIO-RAD). Heart lysates (10μg protein/well) were separated on NuPAGE, 4-12% gradient Bis-Tris gels and MES buffer system (Invitrogen) and transferred to polyvinylidene difluoride (PVDF) membrane (0.45 μm pore size; Invitrogen) using a semi-dry transfer apparatus (BIO-RAD). Membranes were cut appropriately at the correct molecular weights to allow the development of the blots with two different primary antibodies per blot such that each blot was exposed to a test antibody and an internal control antibody to insure equal sample loading. The test primary antibodies including anti-Bcl-2 (N-19) rabbit polyclonal sera (Santa Cruz Biotechnology, Inc.) and anti-Bcl-XS/L (M-125) rabbit polyclonal sera (Santa Cruz Biotechnology, Inc.) were used to detect intragraft expression of Bcl-2 and Bcl-xl proteins. Anti-calsequestrin rabbit polyclonal sera (Calbiochem) were used as internal control primary antibody (Kobayashi et al., 1999). Detection of primary antibody binding was performed as previously described (Arp et al., 1996) by exposing washed incubated blots to a polyclonal goat anti-rabbit IgG fraction conjugated to horseradish peroxidase (HRP; Roche Laboratories) and then appropriately developed by exposure to enhance chemiluminescence for HRP-conjugated antibodies (Roche Laboratories).

Statistical Analysis The data were reported as the mean ± SD. Allograft survival among experimental groups was compared using the rank-log test. Histological and immunohistological findings were analyzed using the Mann-Whitney U test. Flow cytometric data and western blot data were analyzed using one-way ANOVA. Differences with p values less than 0.05 were considered significant.

Example 2

Presensitization with C3H donor skin graft induces antibody-mediated ACHR in heart allografts of BALB/c recipients.

To develop a suitable small animal model that mimics presensitized patients in the clinic and to study ABMR, a novel, fully MHC-mismatched mouse ABMR model has been developed through presensitization of mouse recipients. In this model, BALB/c recipients were presensitized with C3H donor skin grafts one week prior to heart transplantation from the same donor. Seven days after donor skin presensitization, serum level of anti-donor IgG,

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but not IgM antibody was markedly elevated and reached to a peak level in the presensitized BALB/c recipients (Figure 1A). Heart transplantation from same donor was then performed in these highly sensitized recipients. Without immunosuppression, C3H heart grafts were rapidly rejected in 3.1 ± 0.4 days by ACHR, characterized by severe thrombosis, hemorrhage and infarction (Figure 1B-a). In contrast, same heart grafts in unsensitized BALB/c recipients (with mean survival time, MST of 8.2 ± 0.8 days) show the normal histology on post-operative day (POD) 3 (Figure 1B-b). When compared to unsensitized BALB/c recipients at the same day, heart grafts in presensitized animals revealed massive IgG antibody and complement (C3 and C5) deposition, but minimal $CD4^+$ and $CD8^+$ cell infiltration (Table 1). Furthermore, circulating anti-donor IgG levels in presensitized recipients were significantly higher than those of unsensitized same recipients receiving a heart graft on POD3 ($P< 0.01$, Figure 1C). However, anti-donor IgM remained at very low levels both in circulation (Figure 1C) and in heart grafts (Table 1) and it showed no significant difference between unsensitized and presensitized recipients. In addition, normal levels of complement hemolytic activity were shown in both presensitized and unsensitized heart recipients without treatment (Figure 1D). These data indicate that this is an ideal transplant model to study ABMR in presensitized recipients in which complement plays an important role in the pathogenesis.

Table 1.

Comparison of immunohistological changes of C3H heart allografts in unsensitized and presensitized BALB/c recipients on POD3

Groups	Unsensitized	Presensitized
IgG	1+	4+
IgM	1+	1+
C3	2+	3+
C5	2+	3+
CD4	0	1+
CD8	0	1+

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Grades for immunoperoxidase staining: 0, negative; 1+, equivocal; 2+, weak; 3+, moderate; 4+, intense.

Anti-C5 mAb in combination with CsA and CyP prevents ABMR and achieves indefinite heart allograft survival in presensitized mouse recipients.

Complement has been shown to play an important role in ABMR. However, the inhibitory effect of functionally blocking terminal complement cascade at the C5 level in highly sensitized recipients is unknown. In the study presented herein, the presensitized model was used to study the efficacy of anti-C5 mAb either alone or combined with CsA and /or CyP in prevention of ABMR. As presented in Figure 2A, treatment with either CsA or CyP or the two drugs in combination did not prevent ABMR and grafts were rejected in 3.0 ± 0.0 days, 3.3 ± 0.5 days and 3.5 ± 0.6 days, respectively with typical pathological features of ACHR including intravascular thrombosis and interstitial hemorrhage (Figure 2B-b, c, d), which were indistinguishable from heart grafts in untreated presensitized BALB/c recipients (Figure 2B-a). Anti-C5 monotherapy or combined with CyP was not able to improve graft survival and heart grafts were rejected by ACHR (Figure 2B-e, f) in 3.5 ± 0.6 days and 3.2 ± 0.4 days, respectively (Figure 2A). Although the combination therapy of anti-C5 mAb and CsA, the protocol capable of inducing long-term heart allograft survival in unsensitized animals, marginally prolonged graft survival in this presensitized model, heart grafts were also rejected by severe humoral rejection with vasculitis, thrombosis, hemorrhage and minimal cell infiltration (Figure 2B-g) in 11.9 ± 1.8 days (Figure 2A). In contrast, triple therapy of anti-C5 mAb in combination of CsA and CyP achieved indefinite heart graft survival over 100 days (Figure 2A) in presensitized animals ($P<0.01$ vs. the animals without treatment or treated with either monotherapy or two drugs in combination) with no evidence of rejection (Figure 2B-h). In this presensitized mouse model, as shown in Table 2, only minor intragraft $CD4^+$ and $CD8^+$ cell infiltration was observed in the recipients that rejected their heart grafts within 3 days. However, the number of these T cells was slightly increased if heart grafts survived longer in anti-C5 mAb plus CsA-treated recipients at the time of rejection (POD11) and in triple therapy-treated recipients at early stages of graft survival (e.g. POD11). Furthermore, with continuous treatment of CsA in the triple therapy group, $CD4^+$ and $CD8^+$ cell infiltration was inhibited in long-term surviving heart grafts on POD60 and 100. In addition, moderate intragraft Mac-1^+ cell infiltration, including monocytes and macrophages, was found in untreated and CsA-, CyP- or CsA plus CyP-treated animals,

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while the infiltration of these cells was significantly reduced in anti-C5 mAb treated animals (Table 2). These results indicate that functionally blocking anti-C5 mAb enables the use and efficacy of conventional immunosuppressive agents, thereby preventing ABMR and achieving indefinite heart graft survival in presensitized recipients.

Table 2.

Grades for immunoperoxidase staining of heart allografts

in presensitized mouse recipients at necropsy

Groups	Date for sample collection (POD)	C3	C5	**CD4**	CD8	Mac-1	IgG	IgM
Untreated	3	3+	3+	1+	1+	3+	4+	1+
CsA	3	3+	3+	1+	1+	3+	4+	1+
CyP	3	3+	3+	1+	1+	3+	3+	1+
CsA+CyP	3	3+	3+	1+	1+	3+	3+	1+
Anti-C5mAb	3	3+	0	1+	1+	2+	4+	1+
Anti-C5mAb+CsA	11	3+	0	2+	2+	2+	4+	1+
Anti-C5mAb+CyP	3	3+	0	1+	1+	2+	3+	1+
Anti-C5mAb+CsA+CyP	3	3+	0	1+	1+	2+	3+	1+
Anti-C5mAb+CsA+CyP	11	3+	0	2+	2+	1+	3+	1+
Anti-C5mAb+CsA+CyP	60	3+	0	1+	1+	0	2+	1+
Anti-C5mAb+CsA+CyP	100	3+	2+	0	0	0	2+	1+

Grades: 0, negative; 1+, equivocal; 2+, weak; 3+, moderate; 4+, intense.

Anti-C5 mAb completely inhibits total complement hemolytic activity and local C5 deposition in presensitized recipients receiving a heart allograft.

Anti-C5 mAb was previously shown to block the cleavage of complement protein C5 into the proinflammatory molecules C5a and C5b-9 (Kroshus et al., 1995), and to completely and consistently block terminal complement activity in mice (Wang et al., 1999). In the current study, terminal complement activity was measured by assessing the ability of

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recipient mouse sera to lyse antibody presensitized chicken erythrocytes and was compared at the same time-point (POD3). Treatment of mice with either CsA or CyP or the two drugs in combination had no effect on terminal complement activity, while treatment with anti-C5 mAb either alone or combined with CsA or/and CyP completely inhibited this activity (Figure 3; $P<0.01$, vs. naive and untreated animals, as well as CsA-, CyP-, or CsA plus CyP-treated animals). In addition, sera obtained from anti-C5 mAb treated animals at several earlier time points showed similarly diminished hemolytic activity, suggesting that serum terminal complement was inhibited throughout the treatment period. Furthermore, local C5 deposition in heart grafts was completely prevented in the anti-C5 mAb treated presensitized recipients, but not in untreated, or CsA-, CyP- and CsA plus CyP-treated presensitized animals (Table 2). As predicted, treatment with anti-C5 mAb did not prevent C3 deposition in the grafts (Table 2). These results suggest anti-C5 therapy completely blocks total complement activity after cardiac allografting in highly sensitized recipients.

Long-term surviving heart grafts in presensitized animals are resistant to humoral injury in the presence of low level of anti-donor antibodies and complement- a situation of accommodation.

To further investigate the role of anti-C5 mAb in humoral rejection, anti-donor alloantibody levels were measured in recipient sera by flow cytometry and intragraft antibody deposition by using immunostaining techniques in different groups. Figure 4A shows that on POD3 untreated presensitized BALB/c recipients had high levels of circulating anti-donor IgG antibodies. When presensitized recipients receiving either monotherapy or two drugs in combination, CsA and/or CyP partially down-regulated circulating anti-donor IgG levels, while treatment with anti-C5 mAb either alone or combined with CsA or CyP did not further affect anti-donor antibody levels at the same day. In contrast, with triple therapy of anti-C5 mAb, CsA and CyP, a high level of circulating anti-donor IgG was gradually downregulated and reached a low level on POD60, thereafter remaining at this level until day 100 (Figure 4B). Similar to levels of circulating antibodies in the different treatment groups, Table 2 shows that strong deposition of anti-mouse IgG was present in the rapidly rejected heart grafts of presensitized animals with no treatment or treated with monotherapy or two drugs in combination therapy. Interestingly, with triple therapy, IgG antibody deposition was gradually attenuated to a mild level in the long-term surviving heart grafts on POD 100 (Figure 4C-a, Table 2). In this model, IgM remained at very low levels in either circulation (Figure 4A, B) or transplanted heart grafts (Figure 4C-b, Table 2) in presensitized recipients

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with or without treatment. In addition, treatment with anti-C5 mAb eliminated complement activity to an undetectable level until day 60, followed by a progressive recovery to predepletion levels on POD 100 after discontinuation of anti-C5 therapy in presensitized mouse recipients receiving triple therapy (Figure 4D). Furthermore, intragraft C5 deposition was also detected in 100-day surviving presensitized animals (Table 2). These data demonstrate that ongoing transplant accommodation occurs in triple therapy treated presensitized recipients despite the presence of anti-graft antibodies and complement activation.

Anti-C5 mAb in combination with CsA and CyP reduces the IgG1/IgG2a ratio and leads to a shift in IgG subclass to IgG2b in recipients with accommodated grafts.

To determine whether anti-C5 mAb-based triple therapy would induce a shift in IgG subclass, which may be associated with accommodation, serum levels of anti-donor IgG subclasses of IgG1, IgG2a and IgG2b were compared between untreated recipients and the recipients with accommodated heart graft. Sera from untreated recipients contained predominant IgG1 isotype, indicated by a high ratio of IgG1/IgG2a (Figure 5A). In contrast, a significant reduction in the ratio of IgG1/IgG2a was observed in the recipients carrying accommodated grafts (Figure 5A, $P<0.01$). Furthermore, presensitized recipients with the accommodated heart grafts displayed an increased level of anti-donor IgG2b as compared to the same recipients with rejected grafts (Figure 5B, $P<0.01$). In addition, the pattern of IgG isotypes in the recipients treated with either monotherapy or two drugs in combination is indistinguishable from that of untreated animals. These data indicate that anti-donor IgG1 isotype may be associated with graft rejection, while production of anti-donor IgG2b subclass may function as a protective antibody and plays an important role in the induction of accommodation.

Anti-C5 mAb in combination with CsA and CyP induces intragraft Bcl-2 and Bcl-xl expression in highly sensitized mouse recipients.

To determine whether a causal relationship exists between intragraft expression of protective proteins and graft resistance to humoral injury in this model, western blot analysis was employed to detect proteins of interest in heart graft tissues from highly sensitized mouse recipients. Long-term surviving heart grafts were found to express high levels of Bcl-2 and Bcl-xl proteins on POD100, and these proteins were detected as early as 12 days after heart transplantation in highly sensitized recipients receiving anti-C5 mAb-based triple therapy

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(Figure 6). In contrast, there were no Bcl-2 and Bcl-xl proteins expressed on heart grafts of untreated animals (Figure 6) or animals treated with either monotherapy or two drugs in combination therapy. This result suggests that graft resistance to humoral injury in indefinite surviving animals is associated with the protection provided by Bcl-2 and Bcl-xl proteins in this presensitized model.

Presensitized recipients with an accommodating first heart graft accept a second accommodated heart graft but reject a second naive heart graft from the same donors.

The ability of accommodated grafts to resist rejection has not been tested directly under pathophysiological conditions where naive grafts undergo rejection following allotransplantation. In this model, to determine whether presensitized recipients with an accommodating first heart graft will accept a second accommodated graft but reject a second naive graft, we performed re-transplantation scenarios. After the accommodated C3H heart grafts have survived to the 100-day point, the time at which low levels of alloantibodies were detected (Figure 4B) and complement activity has returned to pretreatment levels (Figure 4D), in presensitized BALB/c recipient treated with anti-C5 mAb-based triple therapy, these recipients received a second heart graft. Specifically, either a naive (Figure 7A) or a 100-day accommodated C3H heart (Figure 7B) from another presensitized BALB/c recipient was transplanted into the neck of the presensitized recipients carrying an accommodating first C3H heart. These recipients rejected a second naive heart at 6.6 ± 1.1 days (Figure 8A) with severe AVR (Figure 8B-a) while the first heart continued to survive. In contrast, when the accommodated hearts that had been already surviving for 100 days in different presensitized mice were used as second grafts, these grafts were accepted by the presensitized recipients carrying an accommodating first heart graft (Figure 8A). There was no sign of rejection in those accommodated second heart grafts 90 days after second transplantation (Figure 8B-b). These data indicate that accommodated grafts become resistant to the effects of anti-donor antibodies and complement that normally mediate allograft rejection in these presensitized recipients. Furthermore, the fact that the host of the accommodated graft rejected a new graft suggests that accommodation involves changes to the graft.

Presensitized recipients being treated with CsA reject accommodated heart grafts.

Another re-transplantation was performed to determine whether accommodation in this presensitized model would be caused by the changes in the grafts and/or the recipients. Specifically, after C3H heart grafts have been accommodated in presensitized BALB/c mice

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for 100 days, the accommodated heart graft will then be re-transplanted into a second presensitized BALB/c recipient being treated with CsA alone (Figure 7C), a therapy that can prevent cellular rejection but cannot prevent accelerated humoral rejection of a fresh C3H heart in presensitized recipients. The accommodated C3H heart grafts were rapidly rejected in CsA treated presensitized BALB/c recipients. After re-transplantation, the pathology in accommodated heart grafts was changed from normal (Figure 9A) to severe ACHR with massive interstitial hemorrhage but few cell infiltrates (Figure 9B). In addition, high levels of anti-donor IgG and normal levels of complement hemolytic activity in these recipients receiving an accommodated C3H heart were similar to those of CsA treated presensitized recipients receiving a naive C3H heart. This result further indicates that accommodation induced by anti-C5 mAb-based triple therapy can originate from mechanisms involving changes not only to the graft, but also to the recipient.

Example 3

Acute Vascular Rejection in a Heart Transplantation Model

Experiments were performed to determine whether inclusion of an inhibitor of formation of terminal complement would attenuate acute vascular rejection and whether the use of such an inhibitor in conjunction with an immunosuppressant would achieve long-term allograft survival. In this set of experiments an anti-C5 monoclonal antibody was used in conjunction with cyclosporin. The model used was an allograft heterotopic heart transplant from C3H mice into BALB/c mice. This model is a stringent acute vascular rejection model with the C3H and BALB/c mice being strongly MHC mismatched. The transplantations and other methods were performed as described in Wang et al. (2003).

Heterotopic Cardiac Transplantation

Intra-abdominal heterotopic cardiac transplantation was performed as previously described by Wang et al. (2003). Briefly, a median sternotomy was performed on the donor, and the heart graft was slowly perfused in situ with 1.0 ml of cold heparinized Ringer's lactate solution through the inferior vena cava and aorta before the superior vena cava and pulmonary veins were ligated and divided. The ascending aorta and pulmonary artery were transected, and the graft was removed from the donor. The graft was then revascularized with end-to-side anastomoses between the donor's pulmonary artery and the recipient's inferior vena cava as well as the donor's aorta and the recipient's abdominal aorta using 11-0 nylon suture. The beating of the grafted heart was monitored daily by direct abdominal palpation.

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The degree of pulsation was scored as: A, beating strongly; B, noticeable decline in the intensity of pulsation; or C, complete cessation of cardiac impulses. When cardiac impulses were no longer palpable, the graft was removed for routine histology. In certain instances, mice in which the graft was still functioning were sacrificed to perform histology.

Results

Mice (male 8-12 week old mice weighing 25 – 30 g) were split into six experimental groups with six to eight mice per group. Transplant occurred on day 0. Histological changes were checked at the endpoint (the endpoint being graft failure) or in some cases a mouse was sacrificed prior to graft failure. The dosage of BB5.1 which was administered (40 mg/kg body weight three times per week) was known from prior studies to completely inhibit terminal complement activity.

Group 1' (control) - mice were administered 0.75 mL of saline intraperitoneally on days -1, 0, 1 and 2. Subsequently these mice were treated with 0.75 mL of saline intraperitoneally three times per week (Monday, Wednesday, Friday) until the endpoint.

Group 2' (cyclosporin A alone) - mice were administered 15 mg/kg body weight of cyclosporin A subcutaneously on a daily basis beginning at day 0 (day of transplant) until the endpoint.

Group 3' (anti-complement antibody alone) - mice were administered the anti-mouse C5 antibody BB5.1 (Frei et al., 1987) at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until the endpoint.

Group 4' (anti-complement antibody until day 14 post-transplant plus cyclosporin A) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intravenously on days -1 through day 14 and were also administered cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until the endpoint. Note that this differs from the other groups in that the BB5.1 was administered intravenously and on a daily basis.

Group 5' (anti-complement antibody until day 28 post-transplant plus cyclosporin A) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until day 28 and were also administered

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cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until the endpoint.

Group 6' (anti-complement antibody chronically until 100 days plus cyclosporin) - mice were administered the anti-mouse C5 antibody BB5.1 at 40 mg/kg body weight intraperitoneally on days -1, 0, 1 and 2 followed by 40 mg/kg body weight administered three times per week (Monday, Wednesday, Friday) until 100 days and were also administered cyclosporin A at 15 mg/kg of body weight on a daily basis beginning at day 0 until 100 days.

The results of this experiment are shown in Tables 3 and 4. Table 3 shows the survival time for the grafts. Table 4 sets forth the histological scores.

Table 3

Allograft Survival

Group (Treatment)	Individual Survival (days)	Mean Survival Time (days)
1'. Saline	8, 8, 8, 8, 8, 9	8.3 ± 0.5
2'. Cyclosporin A	14, 15, 15, 16, 16, 16, 17	15.5 ± 1.1
3'. BB5.1	7, 8, 8, 8, 8, 9	8.0 ± 0.6
4'. BB5.1 until day 14 + cyclosporin A	35, 38, 43, 45, 46, 47	42.3 ± 4.8
5'. BB5.1 until day 28 + cyclosporin A	77, 80, 80, 81, 82	80 ±1.9
6'. BB5.1 until day 100 + cyclosporin A	>100 days (7 mice; one sacrificed for histology)	> 100 days

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Table 4

Median Scores of Histological Changes of Heart Allografts at Necropsy

Groups	Vasc*	Infarc	Lymph	Throm	Hemo	Fibrin	PMN
1'. Saline (endpoint)	3.0	3.0	1.0	4.0	3.0	3.0	3.0
2'. Cyclosporin A (endpoint)	2.0	1.0	2.0	3.0	2.0	2.0	3.0
3'. BB5.1 (endpoint)	2.0	1.0	2.0	2.0	1.0	0.0	0.0
4'. BB5.1 until day 14 + cyclosporin A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
5'. BB5.1 28 days + Cyclosporin A (post-operative day 8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5'. BB5.1 28 days + Cyclosporin A (endpoint)	0.0	0.0	1.0	1.0	2.0	1.0	0.0
6'. BB5.1 until day 100 + Cyclosporin A (post-operative day 100)	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Median scores: 0 - normal; 1- minimum change; 2 - mild change; 3 - moderate change; 4 - marked change. N/A - not available.

*Vasc - vasculitis; Infar - infarction; Lymph - lymphocyte infiltration; Throm - thrombosis; Hemo - hemorrhage; Fibrin - fibrin deposition; PMN - polymorphonuclear cell infiltrate

The results indicate the synergistic effects of using a complement inhibiting drug in addition to an immunosuppressant. In untreated mice the grafts were rejected in about 8 davs. Use of the immunosuppressant cyclosporin A alone on a daily, chronic basis resulted

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in an increase in graft survival until approximately 15 days post-transplant. The use of the anti-C5 antibody BB5.1 to inhibit formation of terminal complement had no effect on its own, graft rejection occurring at 8 days post-transplant as in the control group (Group 1'). The combination of BB5.1 through day 28 post-transplant plus cyclosporin A showed a synergistic effect with graft survival being extended until approximately day 80. A more surprising result is that of Group 5' in which BB5.1 and cyclosporin A were each administered chronically post-transplant. In this case the graft survival was for more than 100 days (as much data as presently available). Additionally, the histological results shown in Table 4 indicate that the administration of both BB5.1 and cyclosporin A protected the graft from changes much better than either BB5.1 or cyclosporin A alone, and that the chronic administration of BB5.1 and cyclosporin A protected the graft to such an extent that even at 100 days post-transplant there were no histological changes seen in the engrafted hearts. A survival time of 100 days in these models is considered to be the gold standard. A survival of 100 days in the model is believed to indicate that there will be an indefinite survival of the allograft. When BB5.1 administration was stopped after 28 days, the grafts were protected but they did begin to show some minimal to mild histological changes by about day 80 which was the time at which graft failure occurred.

The Group 4' mice were treated differently in that they were administered BB5.1 on a daily basis by an intravenous administration. These animals became ill, showing weight loss and urine retention and were sacrificed at a time at which the grafted hearts were still beating although their function had declined. This was the first group of mice studied and it is unknown why these ill effects were seen. These ill effects were not seen when the BB5.1 was administered intraperitoneally with a schedule of three times per week. As seen below in Example 4, daily administration of BB5.1 via an intraperitoneal route did not cause ill effects. Also, intravenous administration was not necessarily the cause of the illness in these animals. Intravenous administration of eculizumab (a human equivalent antibody to BB5.1 in that it binds to human C5) has been successfully administered intravenously without ill effects to humans in a study of PNH (Hillmen et al., 2004). Complement inhibitors may be administered by other routes in addition to intravenous and intraperitoneal, with all such routes being well known by those skilled in the art.

Example 4

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Accelerated Rejection in a Presensitized Heart Transplantation Model

A second set of experiments similar to those of Example 3 was performed but the recipient mouse was presensitized to the donor organ. In these experiments, the presensitization was brought about by prior transplantation of a skin graft. In general, presensitization can occur not only as a result of having received an earlier allograft, but can also be caused by having received multiple blood transfusions or in women who have been pregnant. Besides such presensitization methods, allografts with an ABO mismatch will be rapidly attacked and rejected because of preformed antibodies to the ABO antigens unless steps are taken to prevent such an attack.

Some mice in these studies were administered cyclophosphamide in addition to BB5.1 and/or cyclosporin A. For these experiments BALB/c recipient mice were presensitized with C3H skin grafts one week prior to heart transplantation from the same donor (using the method of Pruitt and Bollinger, 1991). This model is designed to mimic presensitized transplantation in humans, especially in relation to accelerated humoral rejection. Recipient mice were split into eight groups of six to eight mice each. The treatments were as follow.

Group 1" (control) - mice (male 8-12 week old mice weighing 25 – 30 g) were administered 0.75 mL saline intraperitoneally on a daily basis beginning at day -1 and continuing until the endpoint (graft rejection).

Group 2" (cyclosporin A alone) - mice were administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight beginning on day 0 (day of transplant) until the endpoint.

Group 3" (BB5.1 alone) - mice were administered the anti-mouse complement monoclonal antibody BB5.1 at a dose of 40 mg/kg body weight delivered intraperitoneally on a daily basis beginning at day -1 and continuing until the endpoint.

Group 4" (cyclophosphamide alone) - mice were administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 5" (BB5.1 plus cyclosporin A) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until the endpoint. These mice were additionally administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until the endpoint.

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Group 6" (BB5.1 plus cyclophosphamide) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until the endpoint. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 7" (cyclosporin A plus cyclophosphamide) - mice were administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until the endpoint. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1.

Group 8" (BB5.1 plus cyclosporin A plus cyclophosphamide) - mice were administered BB5.1 intraperitoneally at a dose of 40 mg/kg body weight on a daily basis beginning at day -1 and continuing until 100 days. These mice were also administered cyclosporin A subcutaneously at a dose of 15 mg/kg body weight on a daily basis from day 0 until 100 days. These mice were additionally administered cyclophosphamide intravenously at a dose of 40 mg/kg body weight on each of days 0 and 1. Two mice in this group were sacrificed at day 60 for histological studies (no rejection had yet occurred) and the four remaining mice still had not rejected their grafts by day 100.

Additionally a control group of mice which was not presensitized and received only the saline treatment as for Group 1" was tested.

The results of these experiments are shown in Tables 5 and 6. Table 5 lists survival times for the grafts and Table 6 summarizes the histological results.

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Table 5

Allograft Survival

Groups (Treatment)	Individual survival (days)	Mean survival time (days)
No presensitization	8, 8, 8, 8, 8, 9	8.3 ± 0.5*
1". One skin presensitization	3, 3, 3, 3, 3, 3, 3, 4	3.1 ± 0.4
2". Cyclosporin A	3, 3, 3, 3	3.0 ± 0.0
3". BB5.1	3, 3, 4, 4	3.5 ± 0.6
4". Cyclophosphamide	3, 3, 3, 4	3.3 ± 0.5
5". BB5.1 + Cyclosporin A	10, 10, 11, 11, 12, 12, 14, 15	11.9 ± 1.8**
6". BB5.1 + Cyclophosphamide	3, 3, 3, 3, 3, 4	3.2 ± 0.4
7". Cyclosporin A + Cyclophosphamide	3, 3, 3, 4, 4, 4	3.5 ± 0.6
8". BB5.1 + Cyclosporin A + Cyclophosphamide	> 100 days (4 mice)	> 100***

*P < 0.01 group 1" vs. no presensitization

**P < 0.01 group 5" vs. groups 1"-4" and 6"-7".

***P<0.01 group 8" vs. groups 1"-7".

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Table 6

Median Scores of Histological Changes of Heart Allografts at Necropsy

Groups	Vasc*	Infar	Lymph	Throm	Hemo	Fibrin	PMN
No presensitization (endpoint)	3.0	3.0	1.0	4.0	3.0	3.0	3.0
1". One skin presensitization (endpoint)	0.0	4.0	1.0	4.0	3.0	0.0	0.0
2". Cyclosporin A (endpoint)	0.0	4.0	0.0	3.0	3.0	N/A	N/A
3". BB5.1 (endpoint)	2.0	2.0	2.0	2.0	3.0	N/A	N/A
4". Cyclophosphamide (endpoint)	2.0	4.0	0.0	3.0	2.0	N/A	N/A
5". BB5.1 + Cyclosporin A (post-operative day 3)	0.0	1.0	1.0	0.0	0.0	0.0	0.0
5". BB5.1 + Cyclosporin A (endpoint)	2.0	2.0	2.0	2.0	2.0	0.0	0.0
6". BB5.1 + Cyclophosphamide (endpoint)	1.0	3.0	0.0	3.0	2.0	N/A	N/A
7". Cyclosporin A + Cyclophosphamide (endpoint)	0.0	1.0	1.0	2.0	3.0	N/A	N/A
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 3)	0.0	0.0	1.0	0.0	0.0	N/A	N/A
8. BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 12)	0.0	0.0	1.0	0.0	0.0	N/A	N/A
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-	0.0	0.0	1.0	0.0	0.0	N/A	N/A

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operative day 60)							
8". BB5.1 + Cyclosporin A + Cyclophosphamide (post-operative day 100)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Median scores: 0 - normal; 1- minimum change; 2 - mild change; 3 - moderate change; 4 - marked change. N/A - not available.

*Vasc - vasculitis; Infar - infarction; Lymph - lymphocyte infiltration; Throm - thrombosis; Hemo - hemorrhage; Fibrin - fibrin deposition; PMN - polymorphonuclear cell infiltrate

The results shown in Table 5 indicate a difference between the presensitized mouse model and the nonpresensitized mouse model as used in Example 3. The results indicate that in the absence of presensitization, grafts are rejected in approximately 8 days in the absence of treatment with any drugs. Presensitizing the animals causes a more rapid rejection, the rejection of the graft in the presensitized animals being in approximately 3 days in the absence of any drug treatment. Treatment with either BB5.1, cyclosporin A or cyclophosphamide had no effect upon graft survival, with the grafts being rejected in approximately 3-4 days in each of these groups of animals. The combination of BB5.1 and cyclosporin A showed some effect with rejection occurring about day 12. The combination of BB5.1 and cyclophosphamide had no protective effect with rejection occurring about day 3. Similarly the combination of cyclosporin A and cyclophosphamide had essentially no protective effect with rejection occurring at 3-4 days. Very surprisingly, the combination of all three drugs (chronic administration of BB5.1 and cyclosporin plus administration of cyclophosphamide at the time of transplant) showed a highly synergistic effect with all of the mice surviving for more than 100 days. Again, a survival of 100 days in this model is considered to be the gold standard and assumes an indefinite survival.

These results as well as the histological results as shown in Table 6 indicate that the combination of chronic treatment with a complement inhibitor and an immunosuppressant such as cyclosporin A in treating a presensitized mouse results in some attenuation of accelerated rejection. Treatment of these animals additionally with cyclophosphamide at the time of transplant and on the first day after transplant results in a much greater time of survival, no rejection having been seen by at least day 100.

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It will be appreciated that the methods and compositions of the instant disclosure can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the disclosure. Thus, the described embodiments are illustrative and should not be construed as restrictive.

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LIST OF REFERENCES

The publications and other materials used herein to illuminate the background of the disclosure, and in particular, cases to provide additional details respecting the practice, are incorporated herein by reference in their entirety, and for convenience, are referenced by author and date in the text and respectively grouped in the following List of References.

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The contents of all references cited herein are incorporated by reference in their entirety.

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CLAIMS

1. A method to prolong survival of an MHC mismatched allograft in a recipient mammal, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

2. The method of claim 1 wherein said mammal is a human.

3. The method of claim 2 wherein said MHC mismatched allograft is an HLA mismatched allograft.

4. The method of claim 1 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

5. The method of claim 4 wherein said drug which inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

6. The method of claim 5 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

7. The method of claim 5 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

8. The method of claim 5 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

9. The method of claim 5 wherein said antibody fragment is pexelizumab.

10. The method of claim 5 wherein said whole antibody is eculizumab.

11. The method claim 10 wherein said eculizumab is administered once every 2 weeks.

12. The method of claim 1 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

13. The method of claim 1 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

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14. The method of claim 1 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

15. The method of claim 1 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

16. The method of claim 1 wherein more than one immunosuppressive drug is administered.

17. The method of claim 1 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

18. The method of claim 17 wherein said drug which inhibits complement activity is an antibody which inhibits cleavage of complement C5.

19. The method of claim 1 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

20. The method of claim 1 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

21. The method of claim 1 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

22. The method of claim 1 wherein said allograft survives for the remaining life-time of said mammal.

23. The method of claim 2 wherein said allograft survives for at least six months.

24. The method of claim 2 wherein said allograft survives for at least one year.

25. The method of claim 2 wherein said allograft survives for at least five years.

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26. The method of claim 2 wherein said allograft survives for the remaining life-time of said human.

27. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

28. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

29. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

30. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

31. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

32. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

33. The method of claim 1 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

34. The method of claim 1 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

35. The method of claim 34 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

36. A method to prolong survival of an allograft in a recipient mammal presensitized to said allograft, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

37. The method of claim 36 wherein said mammal is a human.

38. The method of claim 36 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

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39. The method of claim 38 wherein said drug that inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

40. The method of claim 39 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

41. The method of claim 39 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

42. The method of claim 39 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

43. The method of claim 39 wherein said antibody fragment is pexelizumab.

44. The method of claim 39 wherein said whole antibody is eculizumab.

45. The method claim 44 wherein said eculizumab is administered once every 2 weeks.

46. The method of claim 36 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

47. The method of claim 36 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

48. The method of claim 36 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

49. The method of claim 36 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

50. The method of claim 36 wherein more than one immunosuppressive drug is administered.

51. The method of claim 36 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

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52. The method of claim 51 wherein said method comprises administering i) a drug which inhibits complement activity, ii) cyclosporin A, and iii) cyclophosphamide.

53. The method of claim 51 wherein said drug which inhibits complement activity is an antibody which inhibits cleavage of complement C5.

54. The method of claim 36 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

55. The method of claim 36 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

56. The method of claim 36 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug which inhibits complement activity.

57. The method of claim 36 wherein said allograft survives for the remaining life-time of said mammal.

58. The method of claim 37 wherein said allograft survives for at least six months.

59. The method of claim 37 wherein said allograft survives for at least one year.

60. The method of claim 37 wherein said allograft survives for at least five years.

61. The method of claim 37 wherein said allograft survives for the remaining life-time of said human.

62. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

63. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

64. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

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65. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

66. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

67. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

68. The method of claim 36 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

69. The method of claim 36 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

70. The method of claim 69 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

71. A method to prolong survival of an allograft in a recipient mammal which is an ABO mismatch to said allograft, said method comprising administering to said mammal a) a drug which inhibits complement activity and b) at least one immunosuppressive drug, wherein said drug which inhibits complement activity is administered chronically.

72. The method of claim 71 wherein said mammal is a human.

73. The method of claim 71 wherein said drug which inhibits complement activity inhibits the formation of terminal complement or C5a.

74. The method of claim 73 wherein said drug which inhibits formation of terminal complement or C5a is a whole antibody or an antibody fragment.

75. The method of claim 74 wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

76. The method of claim 74 wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

77. The method of claim 74 wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

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78. The method of claim 74 wherein said antibody fragment is pexelizumab.

79. The method of claim 74 wherein said whole antibody is eculizumab.

80. The method claim 79 wherein said eculizumab is administered once every 2 weeks.

81. The method of claim 71 wherein said inhibitor of complement activity is selected from the group consisting of a i) soluble complement receptor, ii) CD59, iii) CD55, iv) CD46, and v) an antibody to C5, C6, C7, C8, or C9.

82. The method of claim 71 wherein said immunosuppressive drug inhibits T-cell activity or B-cell activity.

83. The method of claim 71 wherein said immunosuppressive drug inhibits T-cell activity and B-cell activity.

84. The method of claim 71 wherein said immunosuppressive drug is selected from the group consisting of cyclosporin A, tacrolimus, sirolimus, OKT3, a corticosteroid, daclizumab, basiliximab, azathioprene, mycophenolate mofetil, methotrexate, 6-mercaptopurine, anti-T cell antibodies, cyclophosphamide, leflunamide, brequinar, ATG, ALG, 15-deoxyspergualin, and bredinin.

85. The method of claim 71 wherein more than one immunosuppressive drug is administered.

86. The method of claim 71 wherein said method comprises administering i) a drug which inhibits complement activity and ii) cyclosporin A.

87. The method of claim 86 wherein said method comprises administering i) a drug which inhibits complement activity, ii) cyclosporin A, and iii) cyclophosphamide.

88. The method of claim 86 wherein said drug that inhibits complement activity is an antibody that inhibits cleavage of complement C5.

89. The method of claim 71 wherein said allograft is selected from the group consisting of i) heart, ii) kidney, iii) lung, iv) pancreas, v) liver, vi) vascular tissue, vii) eye, viii) cornea, ix) lens, x) skin, xi) bone marrow, xii) muscle, xiii) connective tissue, xiv) gastrointestinal tissue, xv) nervous tissue, xvi) bone, xvii) stem cells, xviii) islets, xix) cartilage, xx) hepatocytes, and xxi) hematopoietic cells.

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90. The method of claim 71 wherein said allograft survives for a time at least 20% longer than would occur if said method were to be performed without said drug that inhibits complement activity.

91. The method of claim 71 wherein said allograft survives for a time at least 40% longer than would occur if said method were to be performed without said drug that inhibits complement activity.

92. The method of claim 71 wherein said allograft survives for the remaining life-time of said mammal.

93. The method of claim 72 wherein said allograft survives for at least six months.

94. The method of claim 72 wherein said allograft survives for at least one year.

95. The method of claim 72 wherein said allograft survives for at least five years.

96. The method of claim 72 wherein said allograft survives for the remaining life-time of said human.

97. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 14 days.

98. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 28 days.

99. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 3 months.

100. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 6 months.

101. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 1 year.

102. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for at least 5 years.

103. The method of claim 71 wherein said drug which inhibits complement activity is administered chronically for the remaining life-time of said mammal.

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104. The method of claim 71 wherein at least one immunosuppressive drug is administered chronically for the remaining life-time of said mammal.

105. The method of claim 104 wherein at least cyclosporin A is administered chronically for the remaining life-time of said mammal.

106. Use of a drug that inhibits complement activity and an immunosuppressive drug in the manufacture of a medicament or medicament package for prolong survival of an allograft in a mammal.

107. The use of claim 106, wherein more than one immunosuppressive drug is included in said medicament or medicament package.

108. The use of claim 106, wherein said drug that inhibits complement activity and said immunosuppressive drug are in a formulation suitable for concurrent administration to said mammal.

109. The use of claim 106, wherein said drug that inhibits complement activity and said immunosuppressive drug are in a formulation or formulations suitable for sequential administration to said mammal.

110. The use of claim 106, wherein said drug that inhibits complement activity is in a formulation suitable for chronic administration to said mammal.

111. The use of claim 106, wherein said immunosuppressive drug is in a formulation suitable for chronic administration to said mammal.

112. A method to prolong survival of allograft in a recipient mammal, comprising preparing an allograft from a first allograft accommodated by a first recipient mammal, wherein said first recipient mammal, after receiving said first allograft, has been treated with a drug that inhibits complement activity and at least one immunosuppressive agent.

113. An allograft that has prolonged survival in a recipient mammal, wherein said allograft is prepared from a first recipient mammal and was transplanted to the first recipient mammal that has been treated with a drug that inhibits complement activity and at least one immunosuppressive agent.

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114. A pharmaceutical package comprising a drug that inhibits complement activity and at least one immunosuppressive agent, wherein said drug and said agent are formulated for chronic administration.

115. The pharmaceutical package of claim 114, wherein said drug is an antibody in a lyophilized formulation comprising the antibody and a lyoprotectant.

116. The pharmaceutical package of claim 114, wherein said agent is in a lyophilized formulation comprising the agent and a lyoprotectant.

117. The pharmaceutical package of claim 114, wherein said drug and said agent are in the same lyophilized formulation comprising said drug, said agent, and a lyoprotectant.

118. The pharmaceutical package of claim 114, wherein said drug is in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said drug in a formulation suitable for injection.

119. The pharmaceutical package of claim 114, wherein said agent is in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said agent in a formulation suitable for injection.

120. The pharmaceutical package of claim 114, wherein said drug and said agent are in an injection system comprising a syringe that comprises a cartridge, wherein said cartridge contains said drug and said agent in a formulation suitable for injection.

121. The pharmaceutical package of claim 114, wherein said drug is present in unit dosage form.

122. The pharmaceutical package of claim 114, wherein said agent is present in unit dosage form.

123. A lyophilized antibody formulation comprising an antibody that inhibits complement activity and a lyoprotectant, wherein said antibody formulation is suitable for chronic administration.

124. The lyophilized antibody formulation of claim 123, wherein the antibody inhibits the formation of terminal complement or C5a.

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125. The lyophilized antibody formulation of claim 123, wherein the antibody is a whole antibody or an antibody fragment.

126. The lyophilized antibody formulation of claim 125, wherein the antibody wherein said whole antibody or antibody fragment is a human, humanized, chimerized or deimmunized antibody or antibody fragment.

127. The lyophilized antibody formulation of claim 125, wherein said whole antibody or antibody fragment inhibits cleavage of complement C5.

128. The lyophilized antibody formulation of claim 125, wherein said antibody fragment is selected from the group consisting of an Fab, an $F(ab')_2$, an Fv, a domain antibody, and a single-chain antibody.

129. The lyophilized antibody formulation of claim 125, wherein said antibody fragment is pexelizumab.

130. The lyophilized antibody formulation of claim 125, wherein said whole antibody is eculizumab.

131. An injection system comprising a syringe, wherein said syringe comprises a cartridge containing an antibody that inhibits complement activity and is formulated suitable for injection.

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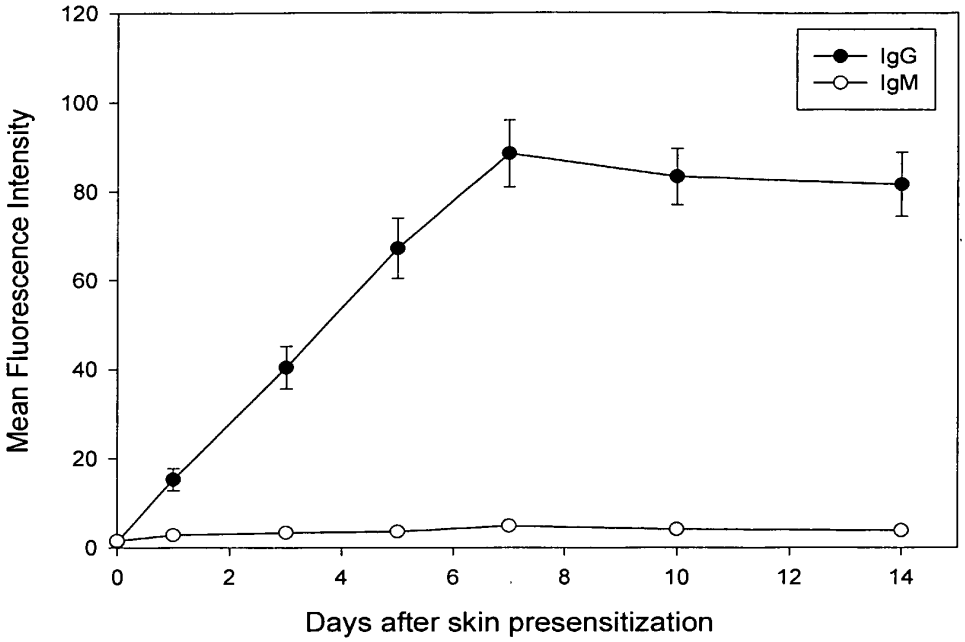
A



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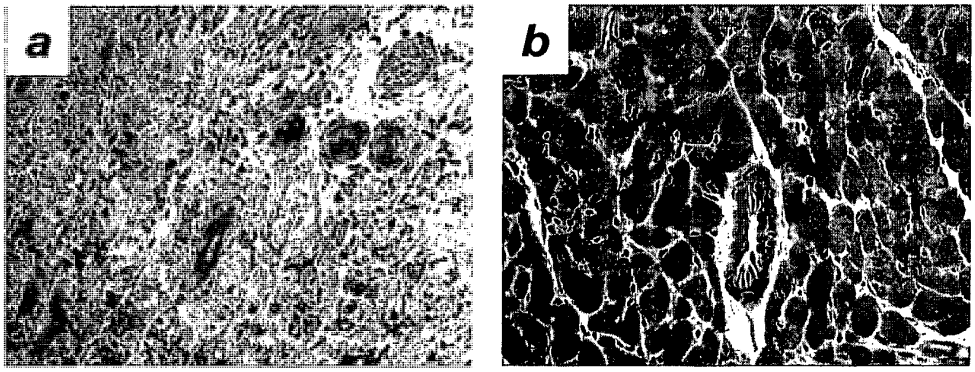

Figure 1A, B

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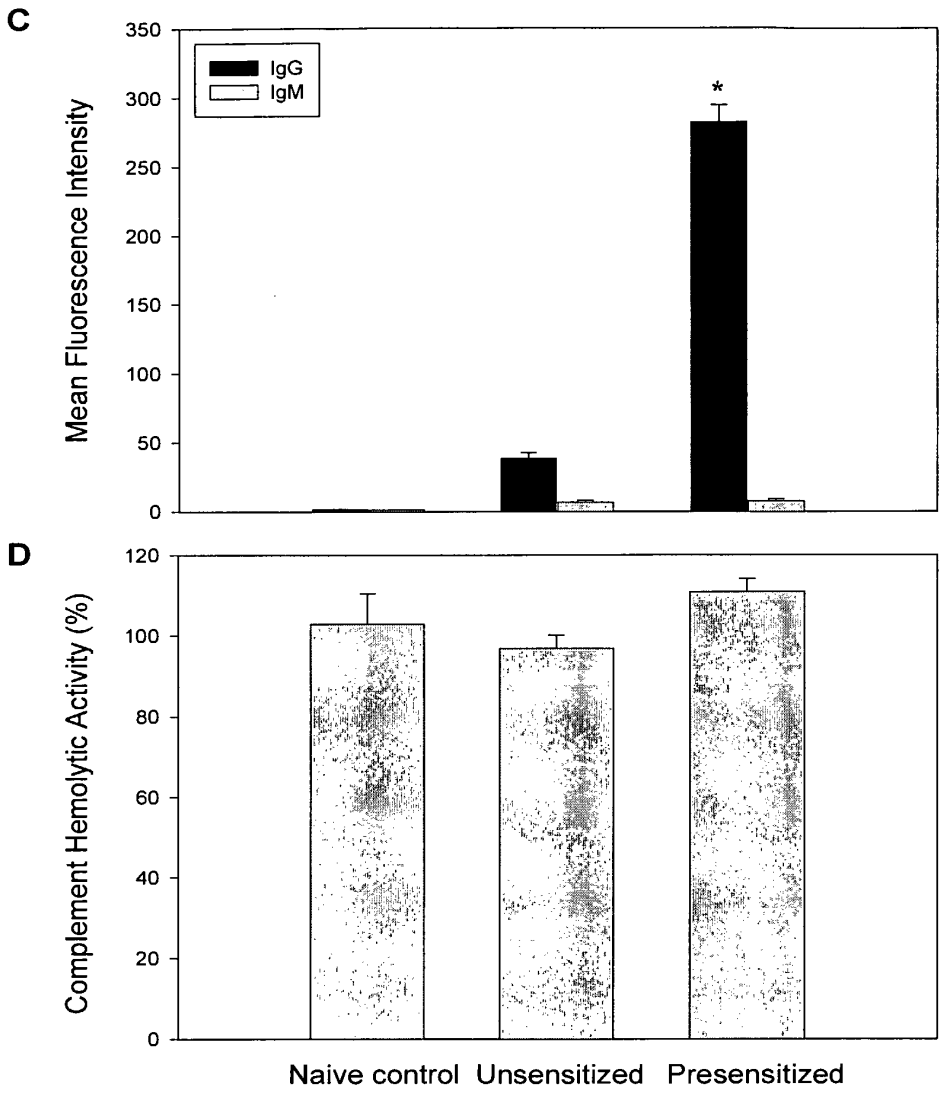

Figure 1C, D

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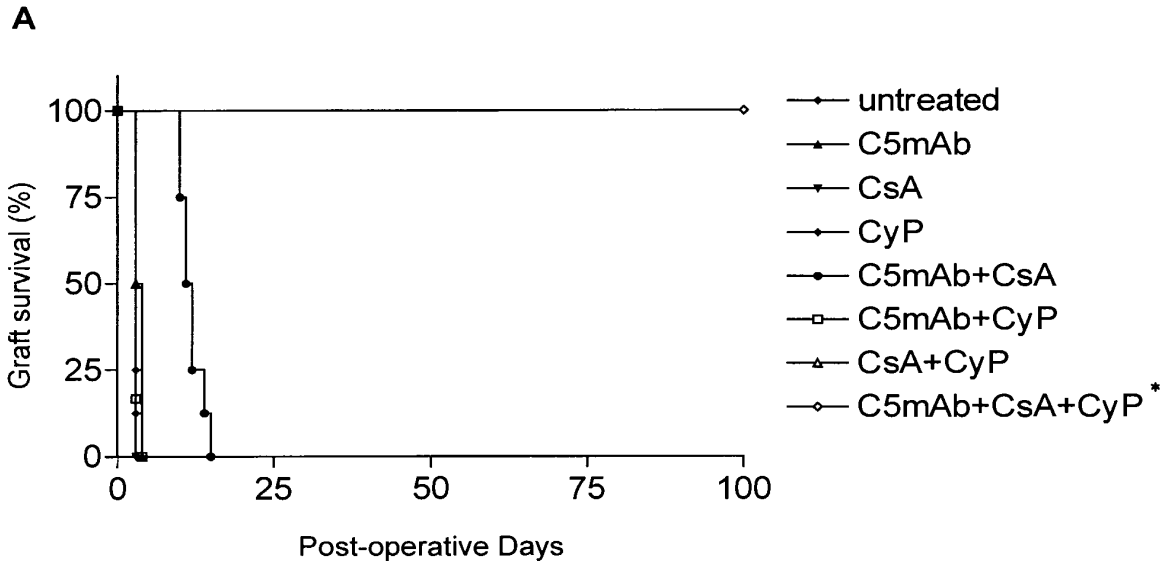

Figure 2A

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B

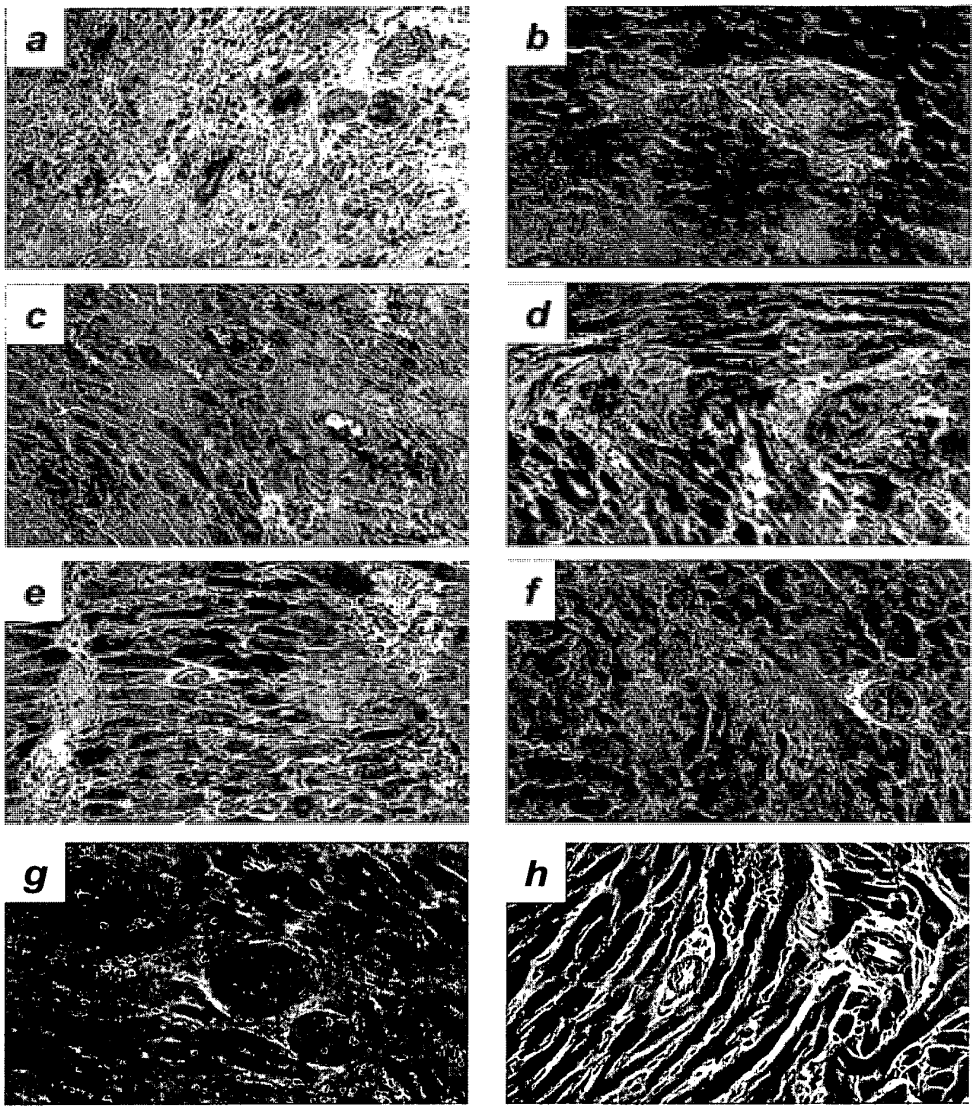

Figure 2B

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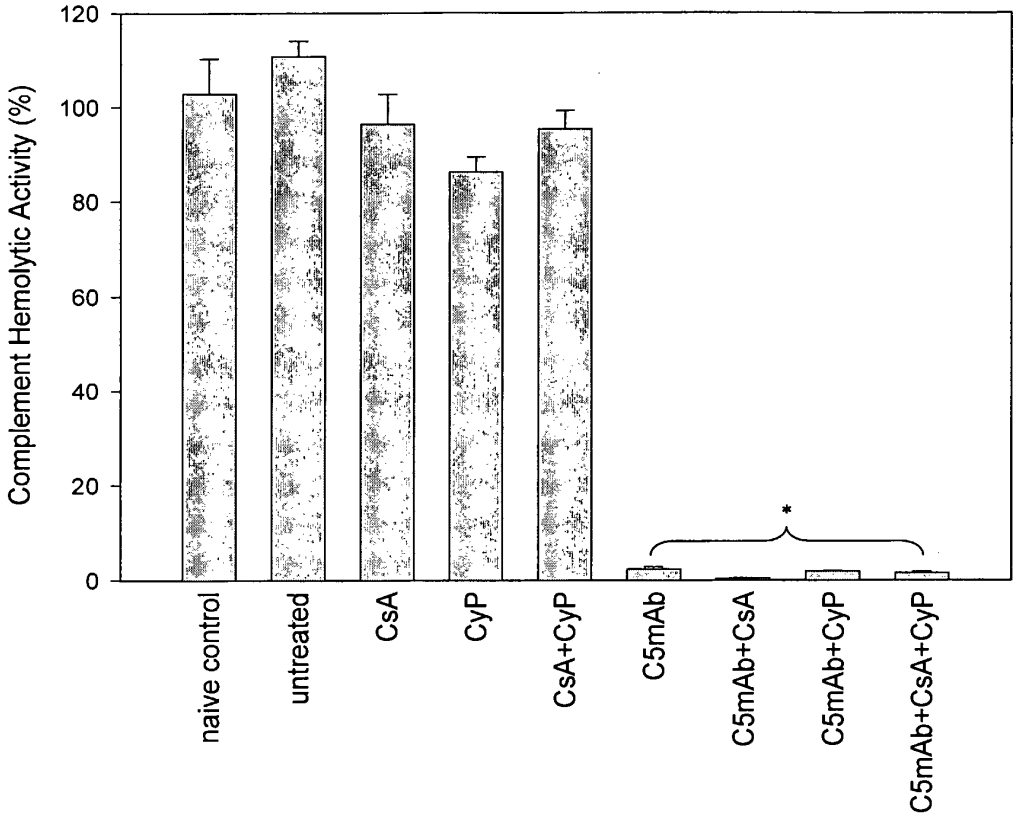

Figure 3

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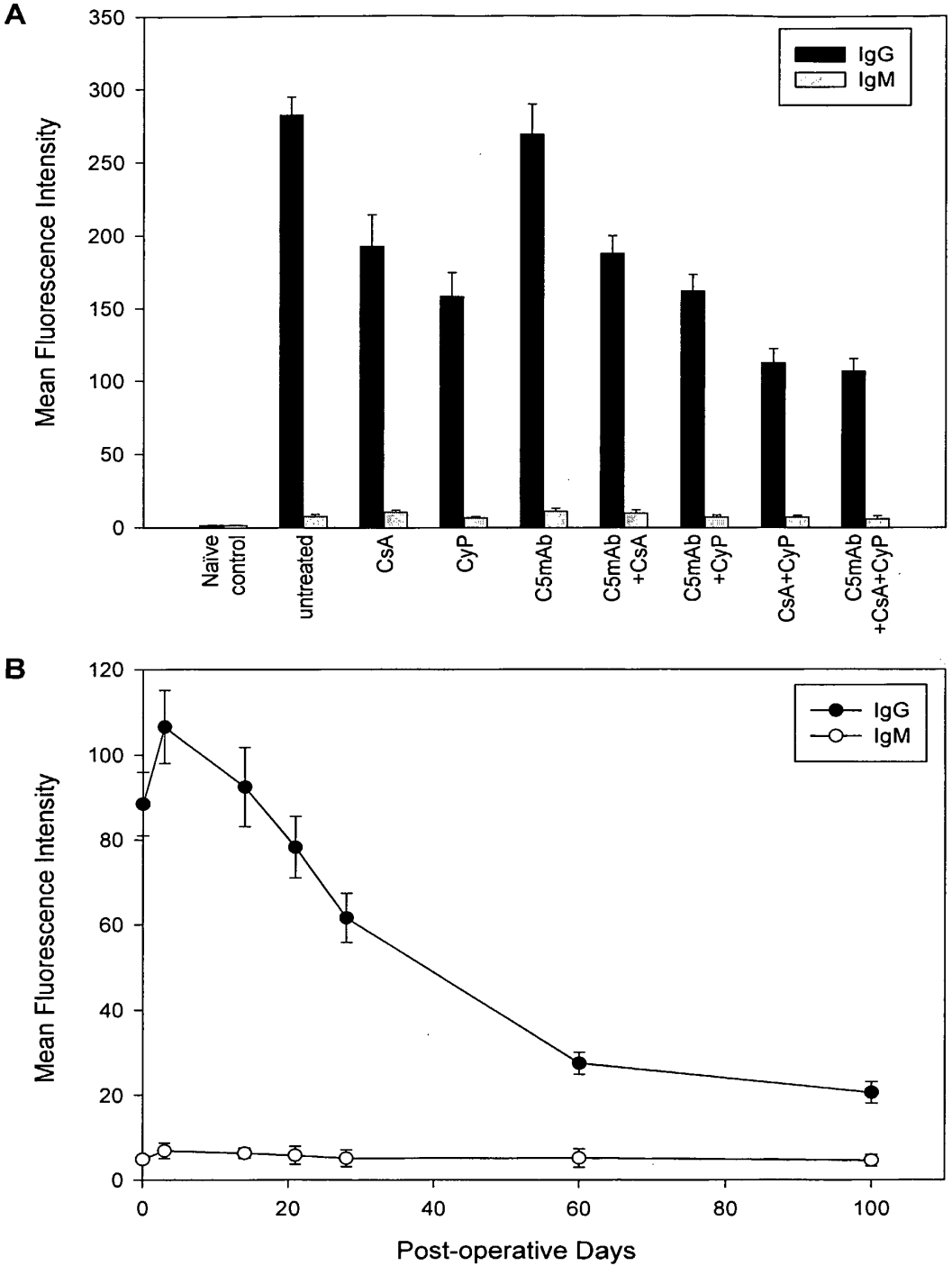

Figure 4A, B

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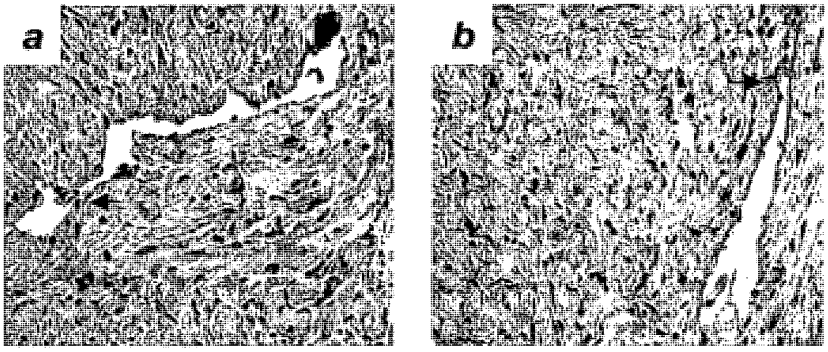
C



D

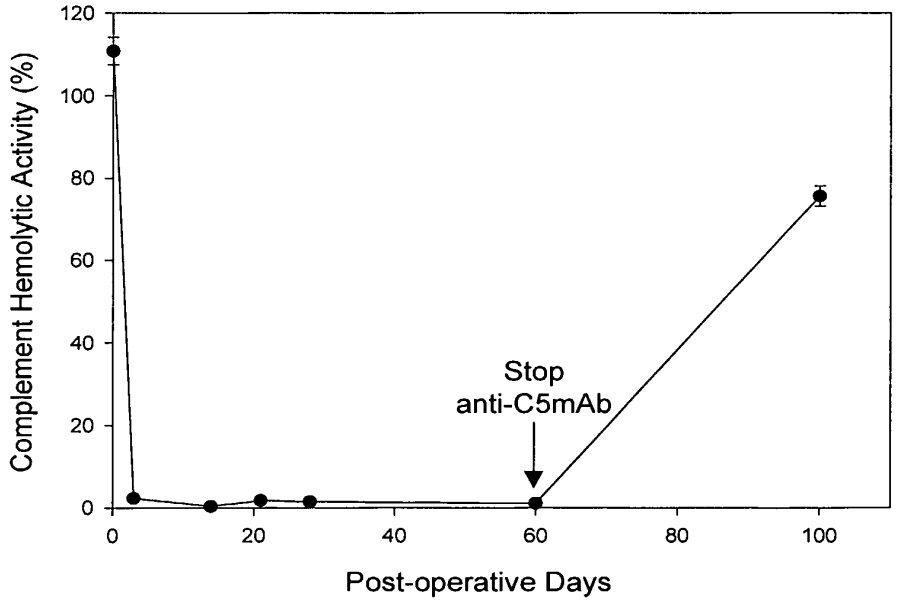


Figure 4C, D

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Page 1227

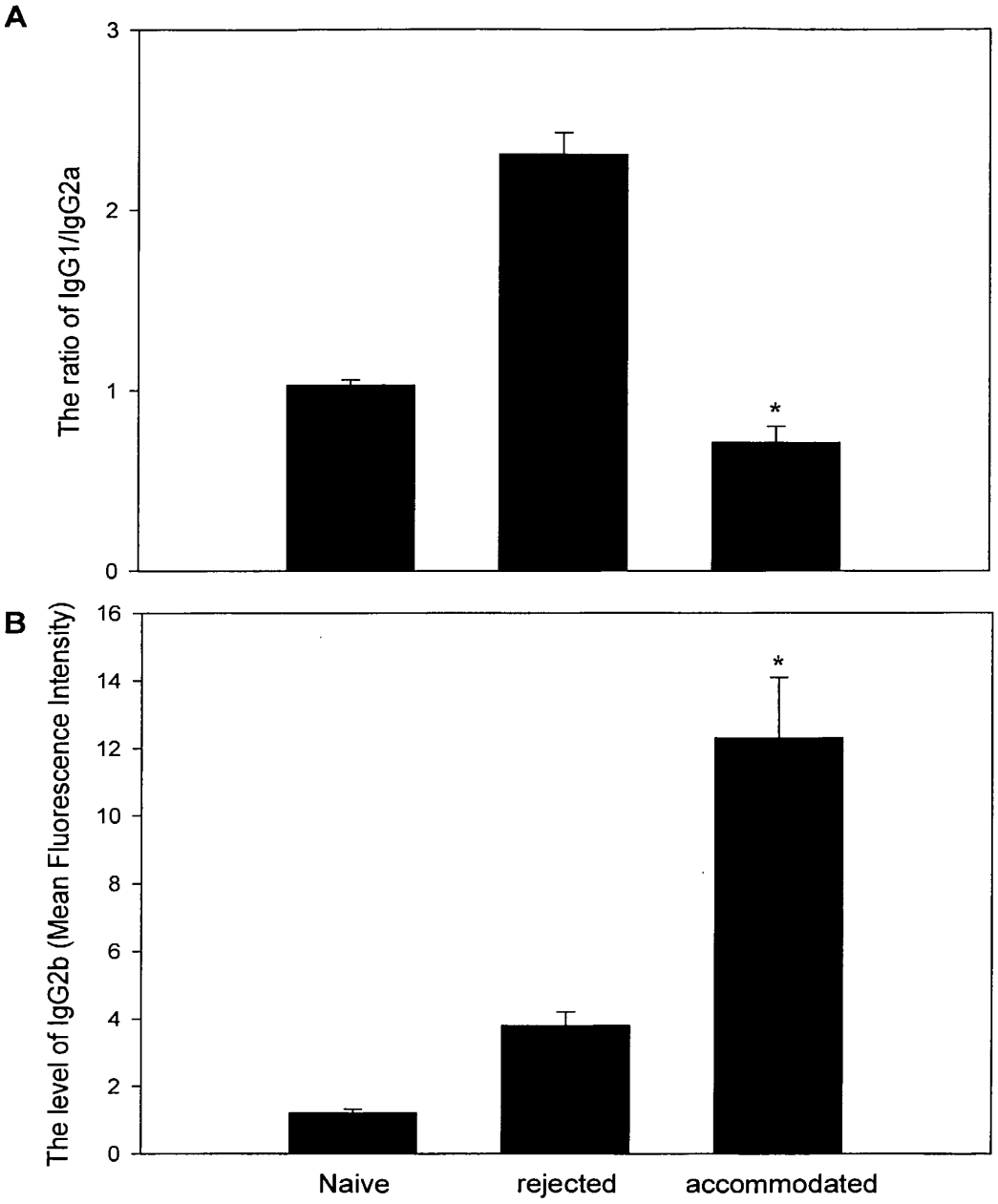

Figure 5A, B

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Page 1228

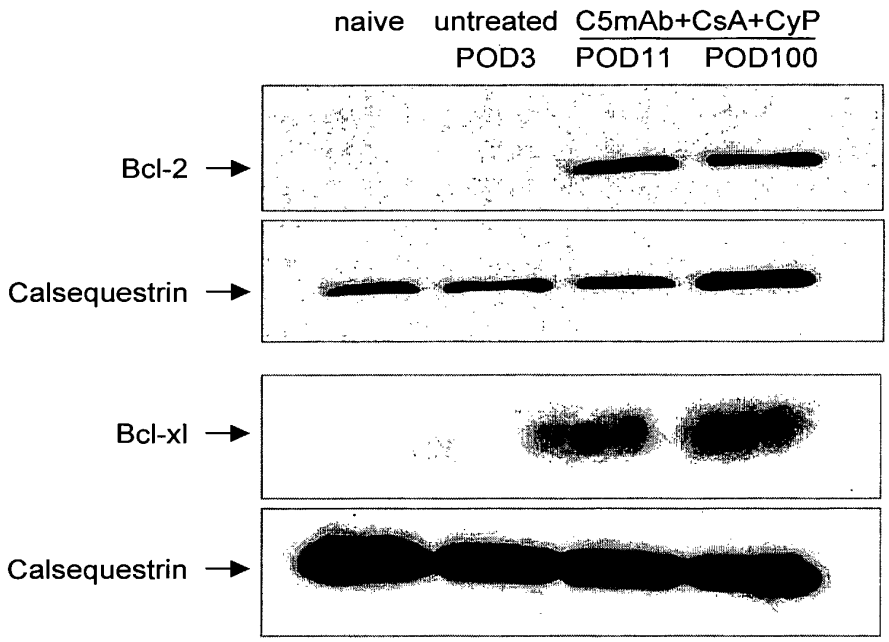

Figure 6

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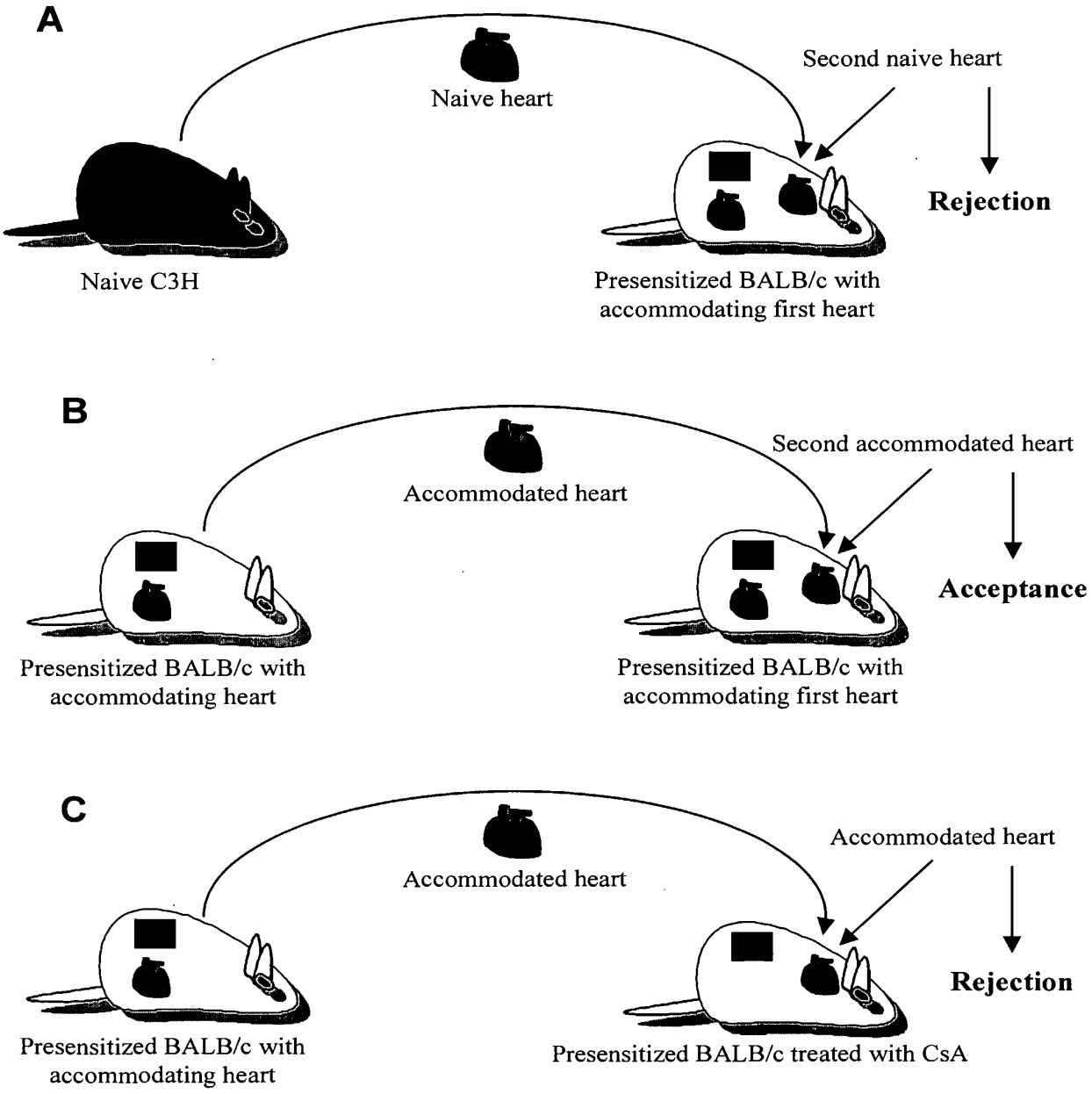

Figure 7

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Page 1230

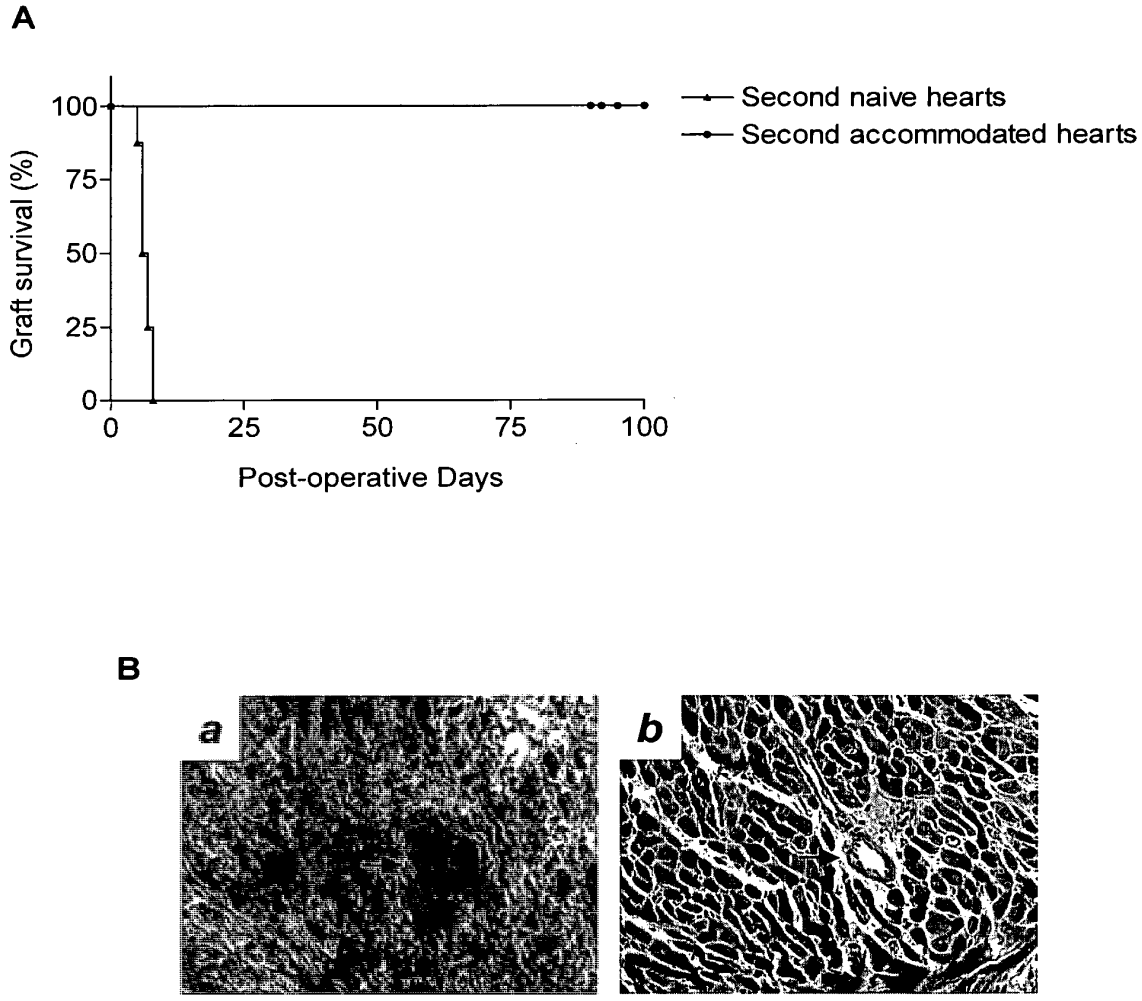

Figure 8

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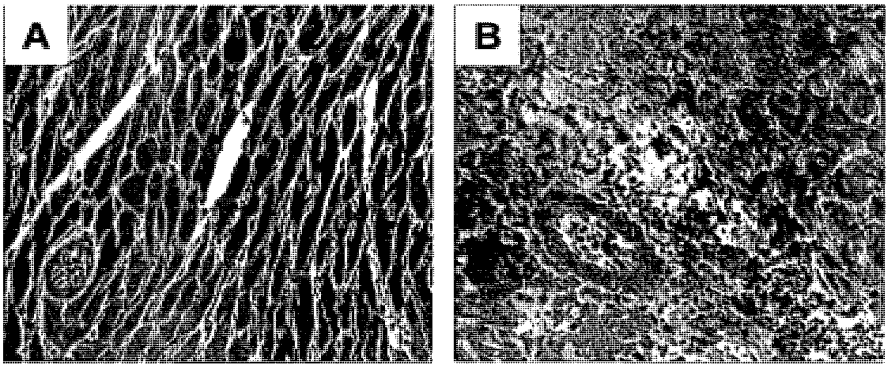

Figure 9

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ClinicalTrials.gov archive
A service of the U.S. National Institutes of Health — Developed by the National Library of Medicine

← History of this study ↑ Current version of this study

View of NCT00098280 on 2005_06_23

ClinicalTrials Identifier: NCT00098280
Updated: 2005_06_23

Descriptive Information

Brief title Eculizumab to Treat Paroxysmal Nocturnal Hemoglobinuria

Official title A Hemoglobin Stabilization and Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria Patients

Brief summary

This study will examine the safety and effectiveness of the experimental drug eculizumab in treating patients with paroxysmal nocturnal hemoglobinuria (PNH), a rare disorder of red blood cells that leads to premature destruction of the cells and resulting anemia. Patients may be at high risk of blood clots and may develop bone marrow failure or aplastic anemia, with low white blood cell and platelet counts. Eculizumab is a monoclonal antibody that may help improve the survival of red blood cells.

Patients 18 years of age and older with PNH who require blood transfusions for anemia and have received at least four transfusions in the 12 months preceding evaluation for this study may be eligible to enroll. Candidates are screened with a medical history, physical examination, and check of vital signs.

Participants have an electrocardiogram (EKG) and blood and urine tests, and are vaccinated against Neisseria meningitides, a common bacteria that can cause a disabling or fatal type of meningitis. They then enter an observation phase of the study, with monthly visits during which they complete a questionnaire; update their health status, transfusion record, and medication use; have their vital signs checked and PNH symptoms evaluated; have blood and urine tests; and receive a transfusion, if necessary. These visits continue for up to 3 months until patients receive a "qualifying" transfusion; that is, a transfusion given as a consequence of a certain hemoglobin level with symptoms or a different level without symptoms.

Patients are then randomly assigned to receive either eculizumab or a placebo (salt solution with no active ingredient). Both study medications are given intravenously (through a vein) over 30 minutes once a week for five doses and then once every 2 weeks for another 11 doses. At each treatment visit (study weeks 0-24), patients update their health status, transfusion records, and medication use; have their vital signs checked; and provide a blood sample. At various visits, they also complete a questionnaire, provide a urine sample and have an EKG. At the last treatment visit (week 26 or the final visit for patients who end their participation before visit 18) patients have a complete physical examination in addition to the procedures listed above.

Detailed description

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disorder of the hematopoietic stem cell characterized by intravascular hemolysis, hemoglobinuria,

anemia, and thrombosis. The clinical features of PNH result from the lack of one or more of the GPI-linked proteins that serve to protect cells from autologous complement mediated attack. Two such proteins, CD55 (decay accelerating factor) and CD59 (reactive lysis inhibitor) have been shown to be absent from PNH erythrocytes and platelets as well as other cell types.

Evidence strongly suggests that lack of the terminal complement inhibitor CD59 is responsible for the sensitivity of PNH erythrocytes and platelets to the effects of autologous complement. Since the pathogenesis of PNH is due to the inability of PNH red cells and platelets to inhibit the activation of terminal complement, it is logical to hypothesize that a terminal complement inhibitor could effectively stop the intravascular hemolysis, obviate or lessen the need for transfusions, and possibly decrease the propensity of life threatening thrombosis. Eculizumab is a humanized monoclonal antibody that like CD59 inhibits terminal complement.

This study is a randomized, double-blind, placebo controlled, multi-center study of eculizumab or placebo administered intravenously to approximately 75 PNH patients. The study is designed to evaluate the safety of eculizumab in transfusion dependent patients with paroxysmal nocturnal hemoglobinuria (PNH) and to determine if the administration of this terminal complement inhibitor could provide a safe and effective substitute for CD59 function.

Phase	III
Study type	Interventional
Condition	Hemoglobinuria, Paroxysmal
Intervention	Drug: Eculizumab
Reference	Citation: Hillmen P, et al. Natural history of paroxysmal nocturnal hemoglobinuria. N Engl J Med. 1995 Nov 9;333(19):1253-8. MEDLINE: 7566002
Reference	Citation: Tabbara IA. Hemolytic anemias. Diagnosis and management. Med Clin North Am. 1992 May;76(3):649-68. Review. MEDLINE: 1578962
Reference	Citation: Rosse WF. Phosphatidylinositol-linked proteins and paroxysmal nocturnal hemoglobinuria. Blood. 1990 Apr 15;75(8):1595-601. Review. No abstract available. MEDLINE: 2183885
URL	http://clinicalstudies.info.nih.gov/detail/A_2005-H-0048.html
See also	Detailed Web Page

Recruitment Information

Status	Recruiting
Start date	2004-12-02

Criteria

INCLUSION CRITERIA - Screening Period:

Individuals at least 18 years of age;

Patients must have required at least 4 episodes of transfusions in the 12 months prior to Visit 1 for anemia or anemia-related symptoms;

Presence of a GPI deficient red blood cell clone (type II cells) by flow cytometry of greater than or equal to 10%;

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Patients taking erythropoietin must be on a stable dose for 26 weeks prior to visit 1 and the dose must be expected to remain stable during the Observation Period and the Treatment Phase;

Patients taking immunosuppressants must be on a stable dose for at least 26 weeks prior to Visit 1 and the dose must be expected to remain stable during the Observation period and the Treatment Phase;

Patients taking corticosteroids must be on a stable dose for at least 4 weeks prior to Visit 1. The dose level may be decreased during the study as clinically appropriate. If the corticosteroids need to be increased, they may not be increased above the dose reported at Visit 1;

Patients are allowed to take coumadin, but must be at a stable international normalized ratio (INR) level for 4 weeks prior to Visit 1 and are expected to maintain a stable INR level during the Observation Period and the Treatment Phase;

Patients taking iron supplements or folic acid must be on a stable dose for 4 weeks prior to Visit 3 and be expected to remain stable during Observation Period and the Treatment Phase;

Patients are allowed to take low molecular weight heparin, but must be on a stable dose for 4 weeks prior to Visit 1 and the dose must be expected to remain stable during Observation period and the Treatment Phase;

Patient must be willing and able to give written informed consent;

Patients must be vaccinated against N. meningitidis at Visit 1 or at least 14 days prior to Visit 3;

Patient must avoid conception during the trial using a method that is most appropriate for their physical state and culture.

INCLUSION CRITERIA - Observation Period:

Documented LDH level greater than or equal to 1.5 x upper limit of normal (ULN) either at Visit 1 or during the Observation Period;

Patients who have received one PRBC transfusion during the Observation Period at a hemoglobin value of less than or equal to 9 gm/dL with symptoms or at a hemoglobin value of less than or equal to 7 gm/dL without symptoms, and also within 1.5 gm/dL of the mean hemoglobin pre-transfusion value for the previous 12 months. This transfusion should be in accordance with that patient's individual transfusion hemoglobin algorithm, and occur within 48 hours of the hemoglobin sample that precipitated the transfusion;

Platelet count greater than or equal to 100,000/mm(3) either at Visit 1 or during the Observation Period.

EXCLUSION CRITERIA:

Patients whose mean hemoglobin levels prior to transfusion over the previous 12 months is greater than 10.5 gm/dL;

Absolute Neutrophil count less than or equal to 500/microL;

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Presence or suspicion of active bacterial infection, in the opinion of the Investigator, at Visit 3 or recurrent bacterial infections;

Known or suspected hereditary complement deficiency;

Participation in any other investigational drug trial or exposure to other investigational agent, device, or procedure within 30 days prior to Visit 1;

Pregnant, breast-feeding, or intending to conceive during the course of the study, including the Post-treatment Phase;

History of meningococcal disease;

History of bone marrow transplantation;

Any condition that, in the opinion of the Investigator, could increase the patient's risk by participating in the study or confound the outcome of the study.

Gender	Both
Healthy volunteers	Yes
Expected enrollment	75

Administrative Data

Secondary ID	05-H-0048
Sponsor	National Heart, Lung and Blood Institute (NHLBI)

Samsung Bioepis Exhibit No. 1002
Page 1236

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Apr 11, 2017 03:46:29 AM

Dear PAIR Customer:

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 133485 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

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The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
15260888	CTNF	04/11/2017	AXJ-114BUSCN3
	892	04/11/2017	AXJ-114BUSCN3
	OA.APPENDIX	04/11/2017	AXJ-114BUSCN3
	OA.APPENDIX	04/11/2017	AXJ-114BUSCN3
	OA.APPENDIX	04/11/2017	AXJ-114BUSCN3

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3	2558

133485 7590 05/11/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
05/11/2017	ELECTRONIC

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

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

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

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	15/260,888	BELL ET AL.	
	Examiner	Art Unit	
	JAMES ROGERS	1644	

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

(1) *JAMES ROGERS*.

(2) *DANIEL KOLKER, SPE*.

(3) *JILL SLOPER*.

(4) *JANE REMILLARD*.

Date of Interview: *01 May 2017*.

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

Exhibit shown or demonstration conducted: ☒ Yes ☐ No.
If Yes, brief description: *TABLES RE: Listing of Clinical Trials*.

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: *1*.

Identification of prior art discussed: _____.

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...)

In addition to the above named participants, Phil Gambel was present for the USPTO and Tara Rahemba, Todd Spalding, and Darrel Fontenot from Alexion were also present. All were present in-person except James Rogers, who joined by video conference.
A follow up to the interview was conducted on 5/5/2017. All of the same participants were present except for Phil Gambel.

The applicant and examiners discussed the Rule 37 CFR 1.105 Request for Additional Information contained in the last office action, and applicant's proposed response. The examiners stated that a rule 132 declaration would be helpful to place all of the documents into context.

The examiners also noted that while no 35 USC 102(a) is on record, the "known or used by others" provisions of 102(a) require public knowledge or use. Thus, if the examiners determine that there is no 102(b) use after consideration of applicant's proposed response, there will also be no 102(a) use.

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

/JAMES ROGERS/ Examiner, Art Unit 1644	/DANIEL E. KOLKER/ Supervisory Patent Examiner, Art Unit 1644

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.

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

May 11, 2017 03:42:22 AM

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UNITED STATES

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Application	Document	Mailroom Date	Attorney Docket No.
15260888	INTV.SUM.APP	05/11/2017	AXJ-114BUSCN3

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I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: June 13, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888 | Confirmation No.: 2558

Filed: September 9, 2016 | Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT | Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (SIDS)

Dear Sir:

In compliance with 37 C.F.R. § 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the document listed on the attached PTO/SB/08. In accordance with 37 C.F.R. § 1.98(a)(2)(i)-(iv), Applicant has not included copies of U.S. patent publication.

It is respectfully requested that the document listed on the PTO/SB/08 be expressly considered by the Examiner during the prosecution of this application, and that the document be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

Applicant provides herewith copies of Office Actions from Applications previously made of record:

Office Actions (copies enclosed)				
Examiner's Initials	Serial No.:	Date Mailed from USPTO	Examiner	Docket No.
	15/284,015	04-11-2017	J. Rogers	AXJ-114BUSCN4
	15/284,015	11-01-2016	J. Rogers	AXJ-114BUSCN4
	15/148,839	06-07-2017	J. Rogers	AXJ-114BUSCN2
	15/148,839	04-04-2017	J. Rogers	AXJ-114BUSCN2

The Examiner is requested to review the file histories of these applications, including cited references, Office Actions, Responses, etc., and is asked to contact Applicant's Attorney if the Examiner would like the Applicant to supply copies of any or all of the information included in these applications. If Applicant's Attorney is not contacted by the Examiner with such a request, then it will be concluded that the Examiner has reviewed or will review the file content of these applications.

Applicant respectfully requests that the Examiner initial the blank columns next to the cited Office Actions, to indicate that the information has been considered by the Examiner. Alternatively, Applicant requests that the Examiner insert the phrase, "All references considered except where lined through," on each page of the Information Disclosure Statement, along with the Examiner's initials.

The filing of this Information Disclosure Statement is not to be interpreted as a representation that the cited document is material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of the document herein be construed *per se* as a representation that such document is prior art. Moreover, Applicant understands the Examiner will make an independent evaluation of the cited document.

This Information Disclosure Statement is filed after the mailing date of a first Office Action on the merits, but before the mailing date of any of a Final Action under 37 C.F.R.§ 1.113, a Notice

of Allowance under 37 C.F.R. § 1.311 or an action that otherwise closes prosecution in this application (37 C.F.R. § 1.97(c)).

Please charge our Deposit Account No. 12-0080 in the amount of $180.00 covering the fee set forth in 37 C.F.R. § 1.17(p). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3.

Dated: June 13, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

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NOTICE OF ALLOWANCE AND FEE(S) DUE

133485 7590 06/07/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER
ROGERS, JAMES L

ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 06/07/2017

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

TITLE OF INVENTION: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

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	09/07/2017

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.

PTOL-85 (Rev. 02/11)

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Page 1245

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)

133485 7590 06/07/2017
Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

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.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

TITLE OF INVENTION: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

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	09/07/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
ROGERS, JAMES L	1644	424-133100

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

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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.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

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EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
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DATE MAILED: 06/07/2017

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

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

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

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

PTOL-85 (Rev. 02/11)

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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. 15/148,839	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	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 *5/12/2017*.
   ☐ 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 *1-3*. 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 5/12/2017
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☒ Examiner's Amendment/Comment
6. ☒ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/JAMES ROGERS/ Examiner, Art Unit 1644	/DANIEL E. KOLKER/ Supervisory Patent Examiner, Art Unit 1644

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DETAILED ACTION

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

2. Applicant's Response, filed 5/12/2017, is acknowledged.

3. Applicant's IDS, filed 5/12/2017, is acknowledged and has been considered.

REASONS FOR ALLOWANCE

4. The following is an Examiner's Statement of Reasons for Allowance:

The claims are drawn to a pharmaceutical composition for use in treating a patient afflicted with paroxysmal nocturnal hemoglobinuria (PNH) where the composition is a sterile, preservative free, 300 mg single use dosage form comprising 30 ml of a 10 mg/ml antibody solution and wherein the antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

Applicant's arguments in the Response of 5/12/2017 to the outstanding Non-final OA of 4/4/2017 which included rejections under 35 USC 102, 35 USC 103 and double patenting rejections as well as a Request for Additional Information Under 37 CFR 1.105 in conjunction with the Declaration Pursuant to 37 CFR 1.132 by Dr. Laural Boone, dated May 11, 2017, is persuasive to overcome all of the rejections for the following reasons:

(a) None of the applied references in the rejections recite using an antibody which comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4 as currently recited and one of skill in the art would not have been easily guided to making antibodies with these recited sequences.

(b) Applicant's Response to the Request for Additional Information is considered sufficient and persuasive to conclude that each of the clinical trials conducted by applicant referenced in the Request did not constitute "public use" under 35 USC 102(b) of the claimed invention as they were not accessible to the public. As applicant notes in the response, factors which are used to determine if an invention is accessible to the public are set out by the Federal Circuit in *Dey, L.P. v. Sunovion Pharmaceuticals, Inc.*, (No. 2012-1428, Fed. Cir. May 20, 2013). The factual circumstances cited by applicant considered by the examiner as being most persuasive to negate any determination that the referenced clinical trials constituted "public use" is the use by applicant of confidentiality provisions (see p. 11 of the response for the 16 clinical studies subject to interrogatories A-F of the Request and pp. 2-3 and p.5 (including sample confidentiality provision" of the Declaration) for the studies and the fact that neither doctors nor

patients had any knowledge of the currently claimed sequences of the antibody used in the studies (see p. 5 of the Response). Other factual evidence presented by applicant includes the declaration by Laural S. Boone that strict control over the eculizumab drug was exercised for each clinical trial referenced (see p. 6 of the Declaration).

(c) The Double Patenting rejections have been overcome in light of applicant's approved TDs of 5/12/2017 over the '601, '340, '980, '263, '035, '102, '176 and '235 patents and '290, 464 and 491' Patent Applications.

As to the remaining two applications (15/243,386 and 11/127,438), the '386 application is now abandoned. The '438 application, as noted by applicant in the Response, recites an aerosol composition comprising an antibody which does not include the currently claimed sequences.

Accordingly, claims 1-3, are deemed allowable.

5. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel E. Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

May 19, 2017

/JAMES ROGERS/
Examiner, Art Unit 1644

/DANIEL E. KOLKER/
Supervisory Patent Examiner, Art Unit 1644

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.
15/148,839	05/06/2016	Leonard BELL	AXJ-114BUSCN2	3159

133485 7590 04/04/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
04/04/2017	ELECTRONIC

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

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

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

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 15/148,839	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	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* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

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

Status

1)☒ Responsive to communication(s) filed on *2/15/2017*.

☐ 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) *1-3* 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) *1-3* 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/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

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

4) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-3 are pending and under consideration in the instant examination and read on a compositions which is a 300 mg eculizumab single-use dosage form comprising 30 ml of a 10 mg eculizumab/ml sterile, preservative free solution.

2. Applicant's two IDSs submitted on 2/15/2017 are acknowledged and have been considered.

Priority

3. The examiner acknowledges applicant's corrected priority claim to 3/15/2007. A Corrected Filing Receipt was mailed from the office on 2/21/2017, Reflecting that the earliest priority date for which priority is claimed is 3/15/2007, the filing date of PCT /US2007 /006606.

Claim Rejections - 35 USC§ 102

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

A person shall be entitled to a patent unless -
(b) the invention was patented or described in a *printed publication in this or* a *foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.*

5. Claim 2 is rejected under 35 U.S.C. 102(b) as being anticipated by Hillmen et al. (N Engl J Med 2004; 350: 552-9, IDS Ref. of 10/21/2016), as is evidenced by applicant's specification as filed at p.44 and Thomas et al. (Molecular Immunology, 33(17/18), pp.1389-1401, 1996).

Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd paragraph).

While Hillmen does not specifically recite that the antibody that binds CS comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, the specification as filed at p. 44 discloses that SEQ ID NO: 2 and SEQ ID NO: 4 constitute the H and L chains for "eculizumab" and Hillmen, as noted supra, teaches administering "eculizumab". Hillmen further teaches that "eculizumab" is a recombinant humanized antibody that binds to C5 (p. 553, 3rd ~) and cites Thomas which in turn describes in detail humanization of the clone referred to as "5G1 .1" arrive at the humanized h5G1 .1 which retain both the affinity and blocking functions of the murine 5g1 .1 antibody (abstract). Accordingly and in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be

inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Hillmen.

The reference teachings anticipate the claimed invention.

To the extent that applicant will argue that Hillmen names eculizumab but does not teach the precise sequences being currently claimed, please note the following:

35 USC 102(b) states:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States,

While it is true that if the antibody was unknown prior, then a reference which names it does not enable that antibody. See MPEP 2121.02 sections I and II. See also 2121.01, "the stated test is whether a reference contains an 'enabling disclosure' " In re Hoeksema, 399 F.2d 269, 158 USPQ 596 (CCPA 1968). The disclosure in an asserted anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation." And ... "Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his [or her] own knowledge to make the claimed invention." In re Donohue, 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985)."

In the instant case, applicant's position is that the mere naming is insufficient. However, the extensive number of both patent literature and NPL documents as well as clinical trials indicates that eculizumab was widespread and well-known. As evidence of the availability and use of eculizumab the examiner refers applicant to the following documents, some of which are part of the Request for additional Information which is also part of this OA:

A) Exhibit A: from clinicalTrials.govClinicaltrials.gov (studies 41-60).

B) Exhibit B: (printout for NCT00005571), in particular page 3 indicating that the trial was ongoing in Bethesda MD, and bottom of page 1 indicating that the drug used was eculizumab.

C) Exhibit C: Clinicaltrials.gov from 6/23/2005 for NCT00098280

D) Exhibit D: Kaplan 2002. Current Opinion in Investigational Drugs 3(7): 10107-1023

E) Exhibit E: Appel et al. Kidney International 2006 70:S45-S50, in particular the paragraph spanning pp. S48-S49.

F) Wang (US 2005/0271660). The reference is a US Patent Application Publication claiming methods of using eculizumab (see claim 7), and is presumed to be enabled.

G) Rother (WO 2005/110481). See for example claims 10, 44-45, 79-80, 123, 125, 130 as well as page 17 line 26 - p. 18 line 12 (noting that the antibody from US Patent 6355245 is now named eculizumab).

H) Rother (US 2009/0028850). This is the publication of the national-stage entry of the '481 application above. Note that the underlying application (11/596382) contains claims to methods of using eculizumab; the office has considered these claims to be enabled. Note that the case recently was decided by the PTAB, and the Board agreed that claims drawn to administering eculizumab were obvious (and thus presumably enabled and operative) as of the filing date of that underlying application, 5/16/2005.

In re Antor Media Corp (CAFC, 2012) states that subject matter disclosed in a reference, whether it is a patent claim, other parts of a patent, or non-patent literature, is presumed to be enabling absent evidence to the contrary. Accordingly, it is the examiner's position that eculizumab was effectively in the public domain.

6. Claim 2 is rejected under 35 U.S.C. 102(b) as being anticipated by **Appel et al.** (Kidney International 70, S45-S50, 2006).

Appel teaches that eculizumab is a humanized monoclonal antibody that prevents the activation of complement component C5. Appel further teaches a randomized trial in 200 patients with eculizumab (S48 and spanning to S49)).

While Appel does not specifically recite that the antibody that binds CS comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Appel.

The reference teachings anticipate the claimed invention.

7. Claim 2 is rejected under 35 U.S.C. 102(b) as being anticipated by **Wang** (US2005/0271660).

Wang teaches an eculizumab that inhibits activation of the complement system (¶s11, 67, 171).

While Wang does not specifically recite that the antibody that binds C5 comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Wang.

The reference teachings anticipate the claimed invention.

Claim Rejections -35 USC§ 103

8. 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 even 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.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. Claims 1-3 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillmen et al. (N Engl J Med 2004; 350: 5S2-9), in view of Evans et al. (US 6,355,245 81, issued 3/12/2002), Wang (US 200S/0271660), as is evidenced by the parent specification of US Patent Application No. 13/426,973 at p. 16, lines 20-25.

Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶). Hillmen teaches that patients received infusions of eculizumab weekly for four weeks, followed one week later by a 900 mg dose and then by a dose of 900 mg every other week through week 12 (p. 554 under "treatment schedule").

The prior art references differ from the claimed invention in the recitation that the composition is a 300 mg eculizumab single-use dosage form comprising 30 ml of a 10 mg eculizumab/ml sterile, preservative free solution as in claims 1 and 3.

However, Evans teaches antibody 5G1.1, which is the same as eculizumab (see parent specification US Patent Application No. 13/426,973, p. 16 lines 20-25), is effective at doses of 2.2, 3, or 5 mg per kg body weight; this corresponds to 154, 210, or 350 mg of antibody for a 70kg adult human ('245 patent, column 16 lines 10-30). Evans also teaches that eculizumab is therapeutically effective when as little as 210 mg are present (column 16 lines 10-15). Evans teaches that the antibodies are particularly advantageous in that they are highly active even when administered in low doses (col 8 lines 25-32), providing a

reasonable expectation of success in treating PHN with a dose less than what was disclosed by Hillmen. The reference teaches that it is within the skill of an artisan, particularly a physician, to determine the optimal dose (column 18 lines 1 5-30). Evans teaches formulations of the anti-C5 antibodies may be formulated in a pharmaceutically effective carrier such saline, which is considered to be a "preservative free solution". (col 18 lines 30-45).

However neither Hillmen nor '245 teaches a 300 mg single unit dosage as recited in the claims.

Nevertheless it would have been obvious to one of ordinary skill in the art to modify the teachings of Hillmen to administer 300 mg single unit dosages. Hillmen teaches that both 600 and 900 mg dosages can be used, and since Evans teaches that 210 or 350 mg of antibody would be expected to be therapeutically effective, arriving at the 300 mg dosage would be routine, convenient and obvious. One of ordinary skill in the art would have a reasonable expectation of success, as Evans teaches that the antibody exerts its effect at low doses.

Finally, **Wang** teaches that eculizumab is highly soluble, including at up to 30 mg per ml (paragraph 0171, Figure 10). Wang further teaches that pharmaceutically acceptable carriers include pyrogen-free water may be used in formulation of the antibody (1[71) sterile, preservative free solution). Therefore the artisan of ordinary skill would have had a reasonable expectation of success in making a 10 mg / ml solution of the antibody sterile, preservative free solution.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Applicant's arguments filed on 2/15/2107 were fully considered but were not considered persuasive.

Applicant asserts that prior to the 3/15/2007 effective filing date of the present application, the complete structure of eculizumab was not disclosed in the prior art; nor was it available to the public. Applicant asserts that neither Hillmen, Wang nor Evans teach or suggest the currently claimed sequence.

This argument was not considered persuasive for the following reasons because eculizumab is considered to have been in public use or on sale in this country more than one year prior to applicant's invention for the reasons stated supra in the anticipation rejection. Note the preponderance of evidence indicates that the antibody was in public use. The public availability of eculizumab is accordingly considered sufficient to have placed one of skill in the art in possession of applicant's currently claimed sequences for eculizumab.

Request for Additional Information Under 37 CFR 1.105

10. Applicant and the assignee of this application are required under 37 CFR 1.105 to provide the following information that the examiner has determined is reasonably necessary to the examination of this application.

11. In response to this requirement, please provide answers to each of the following interrogatories eliciting factual information:

(a) Studies #41-60 in the attached Exhibit A provide prima facie evidence that the methods now claimed were in use in this country more than a year before the document Exhibit A, please answer the following questions:

(i) Which is any of these studies disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) Whether any of the researcher(s) at each of the study sites identified in (i) above was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers for the study(s) identified in (i) above acting solely at the direction of the present inventors?

(b) the study entitled "Safety and Effectiveness of h5G 1.1-mAb for Dermatomyositis" listed on the attached Exhibit B please answer the following questions:

(i) Does this study disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) Whether any of the researcher(s) at the study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (i) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(C) For the study entitled "View of NCT00098280 on 2005_06_23" attached as exhibit C, please answer the following questions:

(i) Does this study disclose the same antibody sequences for eculizumab encompassed

by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) Whether any of the researcher(s) the study sites was under any

confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (i) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(D) Exhibit D: "Eculizumab Alexion –Mariana Kaplan: please answer the following questions with respect to the study (445382) reference on p. 1018, 2nd ¶:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at the study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (i) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(E) Exhibit E (Appel "new approaches to the treatment of glomerular diseases), please answer the following questions with respect to the randomized, placebo-controlled trial in 200 patients referenced at the end of p. S48:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(F) With respect to the IDS submitted on 2/15/2017 which lists NPL literature, please answer the following questions:

(i) Do any of the studies referenced by the NPL documents disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) If the answer to (i) is in the affirmative, were any of the researcher(s) at each of the study sites was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(G) On what date was eculizumab as encompassed by the current claims (i.e., SEQ ID Nos: 2 and 4) first offered for sale?

12. The applicant is reminded that the reply to this requirement must be made with candor and good faith under 37 CFR 1.56. Where the applicant does not have or cannot readily obtain an item of required information, a statement that the item is unknown or cannot be readily obtained may be accepted as a complete reply to the requirement for that item.

Double Patenting

13. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *Jn re Berg,* 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *Jn re Goodman,* 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi,* 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum,* 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel,* 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).
A timely filed terminal disclaimer in compliance with 37 CFR 1.321 (c) or 1.321 (d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(1)(1) - 706.02(1)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321 (b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/e TD-info-1.jsp.

14. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-19 of **US Patent No. 9,494,601,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '601 claim eculizumab (see claim 10 of the '601) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

15. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-18 of **US Patent No. 8,999,340,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '340 claim eculizumab (see claim 27 of the '340) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

16. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of **US Patent No. 9,409,980,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '980 claim eculizumab (see claim 5 of the '980). The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

17. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 3-14 of **US Patent No. 9,556,263,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '263 claim eculizumab (see claims 2 and 4 of the '263) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml

anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

18. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-22 of **US Patent No. 9,352,035,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '035 claim eculizumab (see claim 1 of the '035) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

19. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-14 of **US Patent No. 9,415,102,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '102 claim eculizumab (see claim 15 of the '102) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

20. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of **US Patent No. 9,409,980,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '980 claim eculizumab (see claim 3 of the '980). The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

21. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent No. 9,447,176,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '176 claim eculizumab (see claim 1 of the

'176) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

22. Claims 1-3 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-5,7, 9-16 of US Patent No. 9,388,235, in view of Hillmen et al. (N Engl J Med 2004; 3SO: S52-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '23S claim eculizumab (see claim 31 of the '235) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

25. Claims 1-3 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 15-17, 58-59 of US Patent Application No: 11/127,438, in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '438 claim eculizumab (while not recited in the claims it is clearly a preferred embodiment; as the claim covers the product eculizumab this rejection is proper). The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-CS antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

25. Claims 1-3 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent Application No: 15/243,386,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '386 claim eculizumab (see claim 1 of the '386) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5

antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

26. Claims 1-3 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent Application No: 15/243,290,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '290 claim eculizumab (see claim 1 of the '290) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

27. Claims 1-3 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 94, 97-98 of **US Patent Application No: 15/282,464,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245 81, issued 3/12/2002) and Wang (US 2005/0271660).

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '464 claim eculizumab (see claim 94 of the '464) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

28. Claims 1-3 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 129 – 138 of **US Patent Application No: 15/148,491,** in view of Hillmen et al. (N Engl J Med 2004; 350: 552-9), Evans et al. (US 6,355,245, issued 3/12/2002) and Wang (US 2005/0271660).

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '491 claim eculizumab (see claim 129 of the '491) The difference between the two applications concern the dosages (i.e., 30 ml of a 10 mg/ml antibody and 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5

antibody solution) and the limitation "sterile, preservative free" which would be obvious in view of Hillmen, Evans and Wang as discussed supra.

29. No claim is allowed.

30. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270- 5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

March 21, 2017

/JAMES ROGERS/

/DANIEL E. KOLKER/

Supervisory Patent Examiner, Art Unit 1644

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/284,015	10/03/2016	Leonard BELL	AXJ-114BUSCN4	1040

133485 7590 04/11/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

NOTIFICATION DATE	DELIVERY MODE
04/11/2017	ELECTRONIC

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

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

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

ipboston.docketing@nelsonmullins.com
chris.schlauch@nelsonmullins.com
ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 15/284,015	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	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* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

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

Status

1)☒ Responsive to communication(s) filed on *2/1/2017*.

☐ 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) *1* 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) *1* 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/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *2/1/2017*.

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

4) ☐ Other: _____.

DETAILED ACTION

1. Claims 1 is pending and under consideration in the instant examination and reads On an antibody that binds C5 comprising a H chain SEQ ID NO: 2 and a L chain SEQ ID NO: 4.

2. Applicant's two IDSs submitted on 2/1/2017 are acknowledged and have been considered.

3. Applicant has overcome the outstanding double patenting rejection from the prior OA in light of applicant's approved TDs over 15/148,839 and 15/260,888, filed 2/1/2017.

Claim Rejections - 35 USC§ 102

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

A person shall be entitled to a patent unless -
(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by Hillmen et al. (N Engl J Med 2004; 350: 552-9, IDS Ref. of 10/21/2016), as is evidenced by applicant's specification as filed at p.44 and Thomas et al. (Molecular Immunology, 33(17/18), pp.1389-1401, 1996).

Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶).

While Hillmen does not specifically recite that the antibody that binds CS comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, the specification as filed at p. 44 discloses that SEQ ID NO: 2 and SEQ ID NO: 4 constitute the H and L chains for "eculizumab" and Hillmen, as noted supra, teaches administering "eculizumab". Hillmen further teaches that "eculizumab" is a recombinant humanized antibody that binds to C5 (p. 553, 3rd paragraph) and cites Thomas which in turn describes in detail humanization of the clone referred to as "5G1 .1" arrive at the humanized h5G1 .1 which retain both the affinity and blocking functions of the murine 5g1 .1 antibody (abstract). Accordingly and in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Hillmen.

The reference teachings anticipate the claimed invention.

To the extent that applicant will argue that Hillmen names eculizumab but does not teach the precise sequences being currently claimed, please note the following:
35 USC 102(b) states:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States,

While it is true that if the antibody was unknown prior, then a reference which names it does not enable that antibody. See MPEP 2121.02 sections I and II. See also 2121.01, "the stated test is whether a reference contains an 'enabling disclosure' .... " In re Hoeksema, 399 F.2d 269, 158 USPQ 596 (CCPA 1968). The disclosure in an asserted anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation." And ... "Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his [or her] own knowledge to make the claimed invention." In re Donohue, 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985)."

In the instant case, applicant's position is that the mere naming is insufficient. However, the extensive number of both patent literature and NPL documents as well as clinical trials indicates that eculizumab was widespread and well-known. As evidence of the availability and use of eculizumab the examiner refers applicant to the following documents, some of which are part of the Request for additional Information which is also part of this OA:

A) Exhibit A: from clinicalTrials.govClinicaltrials.gov (studies 41-60).

B) Exhibit B: (printout for NCT00005571), in particular page 3 indicating that the trial was ongoing in Bethesda MD, and bottom of page 1 indicating that the drug used was eculizumab.

C) Exhibit C: Clinicaltrials.gov from 6/23/2005 for NCT00098280

D) Exhibit D: Kaplan 2002. Current opinion in Investigation Drugs 3(7): 1017-1023

E) Exhibit E: Appel et al. Kidney International 2006 70:S45-S50, in particular the paragraph spanning pp. S48-S49.

F) Wang (US 2005/0271660). The reference is a US Patent Application Publication claiming methods of using eculizumab (see claim 7), and is presumed to be enabled.

G) Rother (WO 2005/110481). See for example claims 10, 44-45, 79-80, 123, 125, 130 as well as page 17 line 26 - p. 18 line 12 (noting that the antibody from US Patent 6355245 is now named eculizumab).

H) Rother (US 2009/0028850). This is the publication of the national-stage entry of the '481 application above. Note that the underlying application (11/596382) contains claims to methods of using eculizumab; the office has considered these claims to be enabled. Note that the case recently was decided by the PTAB, and the Board agreed that claims drawn to administering eculizumab were obvious (and thus presumably enabled and operative) as of the filing date of that underlying application, 5/16/2005.

In re Antor Media Corp (CAFC, 2012) states that subject matter disclosed in a reference, whether it is a patent claim, other parts of a patent, or non-patent literature, is presumed to be enabling absent evidence to the contrary.

Accordingly, it is the examiner's position that eculizumab was effectively in the public domain.

6. Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by **Appel et al.** (Kidney International 70, S45-S50, 2006).

Appel teaches that eculizumab is a humanized monoclonal antibody that prevents the activation of complement component C5. Appel further teaches a randomized trial in 200 patients with eculizumab (S48 and spanning to S49)).

While Appel does not specifically recite that the antibody that binds C5 comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Appel.

The reference teachings anticipate the claimed invention.

7. Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by **Wang** (US2005/0271660).

Wang teaches an eculizumab that inhibits activation of the complement system (¶s11, 67, 171).

While Wang does not specifically recite that the antibody that binds C5 comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID N0:4, in absence of evidence to the contrary, the currently recited SEQ ID NO: 2 and SEQ ID NO: 4 which constitute the Land H chains of "eculizumab" are considered to be inherent properties of the "eculizumab" anti-C5 humanized monoclonal antibody taught by Wang.

The reference teachings anticipate the claimed invention. Note that the reference claims eculizumab.

Request for Additional Information Under 37 CFR 1.105

8. Applicant and the assignee of this application are required under 37 CFR 1.105 to provide the following information that the examiner has determined is reasonably necessary to the examination of this application.

9. In response to this requirement, please provide answers to each of the following interrogatories eliciting factual information:

(a) Studies #41-60 in the attached Exhibit A provide prima facie evidence that the methods now claimed were in use in this country more than a year before the document Exhibit A, please answer the following questions:

(i) Which is any of these studies disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID NO: 4)?

(ii) Whether any of the researcher(s) at each of the study sites for which eculizumab covered by the current claims in (i) above was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers in (i) above acting solely at the direction of the present inventors?

(b) the study entitled "Safety and Effectiveness of h5G 1.1-mAb for Dermatomyositis" listed on the **attached Exhibit B** please answer the following questions:

(i) Does this study disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) Whether any of the researcher(s) at the study site for any study identified in (i) above was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers for any study identified in (i) above acting solely at the direction of the present inventors?

(C) For the study entitled "View of NCT00098280 on 2005_06_23" attached as exhibit C, please answer the following questions:

(i) Does this study disclose the same antibody sequences for eculizumab encompassed by the claims of the current application (i.e., SEQ ID NO: 2 and SEQ ID: 4)?

(ii) Whether any of the researcher(s) the study site identified in (i) above was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers for any study identified in (i) above acting solely at the direction of the present inventors?

(D) Exhibit D: "Eculizumab Alexion –Mariana Kaplan: please answer the following questions with respect to the study (445382) reference on p. 1018, 2nd ¶:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at the study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (i) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(E) Exhibit E (Appel "new approaches to the treatment of glomerular diseases), please answer the following questions with respect to the randomized, placebo-controlled trial in 200 patients referenced at the end of p. S48:

(i) Whether the trial used the same sequences for eculizumab as currently claimed (i.e., SEQ ID 2 and 4).

(ii) Whether any of the researcher(s) at study site was under any confidentiality agreement?

(iii) What was the nature of such confidentiality agreement in (ii) above?

(iv) Were the researchers acting solely at the direction of the present inventors?

(F) On what date was eculizumab which is encompassed by the current claimed (i.e., SEQ ID NOs: 2 and 4) first offered for sale?

10. The applicant is reminded that the reply to this requirement must be made with candor and good faith under 37 CFR 1.56. Where the applicant does not have or cannot readily obtain an item of required information, a statement that the item is unknown or cannot be readily obtained may be accepted as a complete reply to the requirement for that item.

Double Patenting

11. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *Jn re Berg,* 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *Jn re Goodman,* 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi,* 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum,* 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel,* 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321 (c) or 1.321 (d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(1)(1) - 706.02(1)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321 (b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/e TD-info-1.jsp.

12. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 7, 9, and 10 of **US Patent No. 9,494,601.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '601 claim eculizumab (see claim 10 of the '601)

13. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-18 of **US Patent No. 8,999,340.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '340 claim eculizumab (see claim

9 of the '34 patent0).

14. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of **US Patent No. 9,409,980.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '980 claim eculizumab (see claim 5 of the '980).

15. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-14 of **US Patent No. 9,556,263.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '263 claim eculizumab (see claims 2 and 4 of the '263)

16. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-22 of **US Patent No. 9,352,035.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '035 claim eculizumab (see claim 1 of the '035).

17. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-15 of **US Patent No. 9,415,102.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '102 claim eculizumab (see for example claims 4 and 15 of the '102).

18. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of **US Patent No. 9,409,980**.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '980 claim eculizumab (see claims 3-4 of the '980).

19. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent No. 9,447,176**.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '176 claim eculizumab (see claim 1 of the '176).

20. Claim 1 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-5, 7, and 9-16 of **US Patent No. 9,388,235**.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '235 claim eculizumab (see claim 5 of the '235).

21. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 15-17, 59 of **US Patent Application No: 11/127,438**.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '438 claim eculizumab (note the specification of the ‘438 application clearly encompasses eculizumab as one of the preferred antibodies).

22. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent Application No: 15/243,386.**

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '386 claim eculizumab (see claim 1 of the '386).

23. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-4 of **US Patent Application No: 15/243,290.**

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '290 claim eculizumab (see claim 1 of the '290).

24. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 94 and 97-98 of **US Patent Application No: 15/282,464**.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.
Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '464 claim eculizumab (see claim 94 of the '464)

25. Claim 1 is provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 129 – 138 of **US Patent Application No: 15/148,491**.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both the current application and '491 claim eculizumab (see claim 129 of the '491)

27. No claim is allowed.

28. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Kolker can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

March 21, 2017

/JAMES ROGERS/

/DANIEL E. KOLKER/

Supervisory Patent Examiner, Art Unit 1644

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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133485 7590 11/01/2016

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
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ipqualityassuranceboston@nelsonmullins.com

Office Action Summary	Application No. 15/284,015	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	AIA (First Inventor to File) Status No

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

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Disposition of Claims*

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6)☐ Claim(s) _____ is/are allowed.

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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)).

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1) ☒ Notice of References Cited (PTO-892)

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

4) ☐ Other: _____.

DETAILED ACTION

1. Claim 1 is pending and under consideration in the instant examination.

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

3. Applicant's IDS's, filed 10/21/2016, are acknowledged and have been considered.

Double Patenting

4. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., In re Berg, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); In re Goodman, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); In re Van Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); In re Thorington, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

5. Claim 1 is provisionally rejected on the ground of nonstatutory obviousness- type double patenting as being unpatentable over claim 1 of copending **Application No. 15/148,839**.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Although the conflicting claims are not identical, they are not patentably distinct from each other because the specification of the ‘839 at ¶158 defines eculizumab as having a heavy chain SEQ ID NO: 2 and light chain SEQ ID NO: 4 which is 100% identical in amino acid sequence to the currently claimed SEQ IDs NO: 2 and 4. (see alignment below).

SEQ ID NO: 2 alignment

```
Qy          1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY 60

Qy         61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV 120

Qy        121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ 180

Qy        181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS 240

Qy        241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST 300

Qy        301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT 360

Qy        361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE 420

Qy        421 GNVFSCSVMHEALHNHYTQKSLSLSLGK 448
              ||||||||||||||||||||||||||||
Db        421 GNVFSCSVMHEALHNHYTQKSLSLSLGK 448
```

SEQ ID NO: 4 alignment

```
Qy          1 MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQ 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQQ 60
```

```
Qy          61 KPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNVLNTPLTF 120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          61 KPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNVLNTPLTF 120

Qy         121 GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN 180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         121 GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN 180

Qy         181 SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 236
               |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         181 SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 236
```

6. The closest prior art was regarded to be Hillmen et al. (N Engl J Med 2004; 350: 552-9). Hillmen teaches a method for treating a human patient that suffers from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an effective amount of eculizumab (p. 552, "methods") which specifically binds to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b (p. 553, 3rd ¶). However, Hillmen does not teach the specific anti-C5 antibody recited by the current application. As evidenced by Report from Alexion Pharma K.K. eculizumab has a H and L chain sequence (p. 3 of the Report) which when compared to the H and L chains of the currently recited anti-C5 antibody are not the same (see sequence alignment below ExPASy (userSeq1 is the imputed sequence of eculizumab and userSeq2 is the amino acid sequence for the H chain of the currently recited claims).

```
98.9% identity in 448 residues overlap; Score: 2364.0; Gap frequency: 0.0%

UserSeq1       1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY
UserSeq2       1 QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEILPGSGSTEY
                 ************************************************************

UserSeq1      61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV
UserSeq2      61 TENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSPNWYFDVWGQGTLVTV
                 ************************************************************

UserSeq1     121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
UserSeq2     121 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
                 ************************************************************

UserSeq1     181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS
UserSeq2     181 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS
                 ************************************************************

UserSeq1     241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
UserSeq2     241 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHANKTKPREEQFNST
                 ********************************************  ************

UserSeq1     301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
UserSeq2     301 YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPEPEQVYTLPPSQEEMT
                 ********************************************   *************

UserSeq1     361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE
UserSeq2     361 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE
                 ************************************************************

UserSeq1     421 GNVFSCSVMHEALHNHYTQKSLSLSLGK
```

```
UserSeq2      421 GNVFSCSVMHEALHNHYTQKSLSLSLGK
                  ****************************
```

One of skill in the art would not have been easily guided to using the currently claimed anti-C5 antibody for treating a patient suffering from PNH given the teachings of Hillmen.

7. No claim is allowed.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

October 26, 2016

/JAMES ROGERS/
Patent Examiner, AU 1644

/SHARON WEN/
Primary Examiner, Art Unit 1644

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

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99**)		
	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

CERTIFICATION STATEMENT

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

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

OR

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

See attached certification statement.

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

☒ A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Jill Gorny Sloper, Esq./	Date (YYYY-MM-DD)	2017-06-13
Name/Print	Jill Gorny Sloper, Esq.	Registration Number	60,760

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

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

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

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

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

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

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

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

Electronic Patent Application Fee Transmittal

Application Number:	15260888
Filing Date:	09-Sep-2016
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper/Anita Costa
Attorney Docket Number:	AXJ-114BUSCN3

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

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

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

Electronic Acknowledgement Receipt

EFS ID:	29476501
Application Number:	15260888
International Application Number:	
Confirmation Number:	2558
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3
Receipt Date:	13-JUN-2017
Filing Date:	09-SEP-2016
Time Stamp:	17:42:41
Application Type:	Utility under 35 USC 111(a)

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Warnings:					
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Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30872 fc5d396415088b3f2e3f90e4d6bfa36cd7c54445	no	2
Warnings:					
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New Applications Under 35 U.S.C. 111

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

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

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.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: June 13, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888 Confirmation No.: 2558

Filed: September 9, 2016 Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.111

Dear Sir:

In response to the Office Action dated April 11, 2017 (Paper No. 20170227), please amend the above-identified U.S. patent application as follows:

A Listing of the Claims begins on page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

LISTING OF THE CLAIMS

1. **(Original)** A method of treating a patient suffering from paroxysmal nocturnal hemoglobinuria (PNH) comprising administering to the patient a pharmaceutical composition comprising an antibody that binds C5, wherein the antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

2. **(Original)** The method of claim 1, wherein the pharmaceutical composition is administered by intravenous infusion.

3. **(Original)** The method of claim 1, wherein the antibody is administered to the patient at a dosage level of between 5 mg per kg and 50 mg per kg per patient per treatment.

4. **(Original)** The method of claim 1, wherein the pharmaceutical composition is in a single unit dosage form.

5. **(Original)** The method of claim 4, wherein the single unit dosage form is a 300 mg single unit dosage form.

6. **(Original)** The method of claim 1, wherein the pharmaceutical composition comprises a 300 mg single-use formulation of 30 ml of a 10 mg/ml sterile, preservative free solution.

7. **(Original)** The method of claim 1, wherein the patient is anemic.

8. **(Original)** The method of claim 1, wherein the patient is dosed as follows: 600 mg of the antibody via intravenous infusion every 7±1 days for 4 doses; followed by 900 mg of the antibody via intravenous infusion 7±1 days later; followed by a maintenance dose of 900 mg of the antibody via intravenous infusion every 14±2 days.

9. **(Original)** The method of claim 1, wherein administration of the antibody results in an immediate and sustained decrease in mean levels of lactate dehydrogenase (LDH).

10. **(Original)** The method of claim 9, wherein the immediate decrease occurs within one week of administration of the antibody.

REMARKS

Claims 1-10 are pending.

Interview Summary

Applicants thank the Examiner and his Supervisor for the interview held on May 1, 2017 and follow-up discussion on May 5, 2017, during which the outstanding rejections and related Rule 37 C.F.R. § 1.105 Request for Additional Information in the above-referenced application were discussed. The substance of the interview and follow-up discussion is reflected in the following remarks.

Moreover, as confirmed in the Interview Summary dated May 11, 2017, if the Examiners determine after consideration of the present response and accompanying Declaration (submitted for related Application No. 15/148,839) that there was no 35 U.S.C. § 102(b) "public use" of the claimed invention, then there also was no 35 U.S.C. § 102(a) "knowledge or use" of the claimed invention.

Information Disclosure Statement

Applicants submit herewith a Supplemental Information Disclosure Statement to make of record additional information for the Examiner's consideration.

Rejection of Claims 1-2 and 7-10 Under 35 U.S.C. § 102(b)

The Examiner has rejected claims 1-2 and 7-10 as allegedly being anticipated by Hillmen *et al.* (*N. Engl. J. Med.* 2004; 350; 552-9), as evidenced by Applicants' specification as filed at page 44 and Thomas *et al.* (*Molecular Immunology*, 33 (17/18), pp. 1389-1401, 1996). The Examiner relies on Hillmen *et al.* for teaching a method for treating a human patient that suffers from PNH by administering to the patient an effective amount of eculizumab. The Examiner relies on Applicants' specification at page 44 for disclosing that SEQ ID NOs: 2 and 4 constitute the heavy and light chains of eculizumab. The Examiner relies on Thomas *et al.* for teaching the humanized h5G1.1 antibody, which retains the affinity and blocking functions of its murine counterpart.

The Examiner acknowledges that none of the cited references specifically teaches that eculizumab comprises heavy and light chains consisting of the amino acid sequences set forth in SEQ ID NO:2 and SEQ ID NO:4, respectively. However, the Examiner is of the opinion that, absent evidence to the contrary, these heavy and light chain sequences are "inherent properties" of eculizumab.

Applicants respectfully traverse the rejection. As explained in the Office Action on pages 4-5, the premise of the Examiner's inherency rejection is that eculizumab (and thus, its full length sequence) "was effectively in the public domain", as evidenced by Exhibit A (studies 41-60 from clinicaltrials.gov), Exhibit B (clinicaltrials.gov from 6/23/2005 for NCT00098280), Exhibit C (printout for NCT00005571), Exhibit D (Kaplan 2002), Exhibit E (Appel *et al.* 2006), Exhibit F (Wang, US20050271660), Exhibit G (Rother, WO 2005/110481), and Exhibit H (Rother, US 2009/0028850). However, this is incorrect. Neither eculizumab nor its complete sequence, including the sequence of its unique, non-naturally occurring, protein-engineered heavy chain, was in the public domain prior to the March 15, 2007 effective filing date of the present application, as discussed in detail below. Accordingly, the presently claimed method of treating a patient suffering from paroxysmal nocturnal hemoglobinuria (PNH) comprising administering to the patient a pharmaceutical composition comprising eculizumab (and the specific sequences thereof) was also not in the public domain prior to that time.

A. Exhibits A-C: Clinical Trials

With respect to the clinical trials cited by the Examiner in Appendices A-C, these clinical trials did not constitute "public use" of eculizumab under pre-AIA 35 U.S.C. § 102(b), as they were controlled, restricted, and subject to strict confidentiality obligations. As discussed below in response to the Request for Additional Information under 37 C.F.R. § 1.105, Applicants provide a copy of the (1) Amendment and Response and (2) Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone that were submitted in related U.S. Patent Application No: 15/148,839 ("the '839 application") on May 12, 2017. Specifically, as discussed in the enclosed Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone, the cited clinical trials using eculizumab were strictly controlled and governed by clinical trial protocols which included explicit confidentiality

requirements. The Federal Circuit has clearly held that clinical trials performed under such confidentiality and restrictions do not constitute "public use" within the meaning of 35 U.S.C. § 102(b). In *Dey, L.P. v. Sunovion Pharmaceuticals, Inc.* (No. 2012-1428, Fed. Cir. May 20, 2013) the Federal Circuit considered whether a clinical trial constitutes an invalidating "public use" under 35 U.S.C. § 102(b). Sunovion, the accused infringer, asserted that its own clinical trial was an invalidating public use against Dey's patents, because they involved the accused formulation and took place before Dey's 102(b) critical date. The Federal Circuit concluded that the trial was not an invalidating public use of the study medication based on the following factors: the nature of the activity that occurred in public; the public access to and knowledge of the public use; and whether there was any confidentiality obligation imposed on persons who observed the use (citing *Bernhardt, L.L.C. v. Collezione Europa USA, Inc.*, 386 F.3d 1371, 1379 (Fed. Cir. 2004)). Applying this standard, the Federal Circuit reversed the district court's grant of summary judgment to Sunovion because the clinical trial's restrictions, instructions, and records requirements supported a conclusion ***that the trial was sufficiently controlled and restricted and therefore, not an invalidating public use***. In addition, the Court noted that the ***investigators (i.e., "the most knowledgeable persons involved in the study") were under express confidentiality restrictions, and neither the patients nor the doctors were aware of the claimed particulars of Sunovion's formulation*** (page 10 of *Dey*). Specifically, "while it is true that participants were permitted to discuss the study with their doctors, ***they were not in a position to reveal the composition…because they were unaware of the specifics of the inventive formulations***" (page 10 of *Dey*). As argued by Dey and agreed by the Court, "***if members of the public are not informed of, and cannot readily discern, the claimed features of the invention in the allegedly invalidating prior art, the public has not been put in possession of those features***."

Similar to the facts in *Dey*, the trials cited in Exhibits A-C on page 4 of the Office Action were tightly controlled, restricted and governed by express confidentiality provisions, as discussed in detail in the enclosed Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone. Moreover, similar to the facts in *Dey*, the clinical trial protocols did not contain the specifics of the claimed invention or allow the participants to readily discern the features of the presently claimed invention, including the sequence of the antibody being administered, *i.e.*, eculizumab. Thus, in accordance

with *Dey*, the cited clinical trials did not constitute "public use" of eculizumab under pre-AIA 35 U.S.C. § 102(b).

B. Exhibits D-H: Publications

With respect to the publications cited in Exhibit D (Kaplan 2002), Exhibit E (Appel *et al.* 2006), Exhibit F (Wang, US20050271660), Exhibit G (Rother, WO 2005/110481), and Exhibit H (Rother, US 2009/0028850), the Examiner is of the opinion that these publications demonstrate that "eculizumab was effectively in the public domain" prior to the effective filing date of the present application. In support of this assertion, the Examiner cites *In re Antor Media Corp* (CAFC, 2012) for the proposition that "the subject matter disclosed in a reference, whether it is a ***patent claim***, ***other parts of a patent***, or non-patent literature, is presumed to be enabling absent evidence to the contrary (emphasis added)."

Applicants respectfully disagree with the Examiner's position. From the outset, as noted by the Court in *In re Antor Media Corp*, the question of enablement only arises if the cited reference in fact teaches each and every element of the claimed invention (page 9 of *In re Antor Media Corp*). In the present case, none of the publications cited in Exhibits D-H teach the complete structure of eculizumab, as presently claimed, including its complete sequence with a unique, non-naturally occurring, protein-engineered heavy chain. Nor do these references enable the presently claimed full biochemical structure of eculizumab, for the reasons discussed above. Exhibit D (Kaplan 2002) describes clinical studies that used eculizumab. Exhibit E (Appel *et al.* 2006) merely discloses that eculizumab is a humanized monoclonal antibody that prevents the activation of complement component C5. Exhibit F (Wang, US20050271660) discloses antibody formulations suitable for nebulization using murine antibodies in the examples. Exhibit G (Rother, WO 2005/110481) and Exhibit H (Rother, US 2009/0028850, which was not published until after March 16, 2007) disclose the use of eculizumab for treating PNH patients, as well as the use of the mouse anti-C5 antibody BB5.1 in prolonging the survival of an allograft. Importantly, none of the cited references teach the sequence of eculizumab, let alone its unique, non-naturally occurring, protein-engineered full heavy chain (including the CH1-hinge-CH2-CH3 regions).

As discussed above, to anticipate a claimed invention, a reference must provide an enabling disclosure of the desired subject matter. Specifically, pursuant to M.P.E.P § 2121.1, "***mere naming or description of the subject matter is insufficient,*** if it cannot be produced without undue experimentation. *Elan Pharm., Inc. v. Mayo Found. For Med. Educ. & Research,* 346 F.3d 1051, 1054, 68 USPQ2d 1373, 1376 (Fed. Cir. 2003) (emphasis added)." Further, a "reference contains an 'enabling disclosure' if the public was in possession of the claimed invention before the date of invention." Such "***possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his [or her] own knowledge to make the claimed invention***. *In re Donohue,* 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985) (emphasis added)."

It is clear that the public was not in possession of the claimed sequences of eculizumab prior to the effective filing date of the present application by virtue of the cited publications, because the ***mere naming of eculizumab is insufficient to enable*** the claimed sequences and ***one of ordinary skill in the art could not have readily determined the sequences based on the limited disclosure in the publications and his or her own general knowledge***.

C. Conclusion

In sum, none of the cited references teach or enable the complete sequence of eculizumab, as presently claimed. Moreover, eculizumab was not in "public use" under 35 U.S.C. § 102(b) prior to the March 15, 2007 effective filing date of the present application, at least because the clinical trials cited by the Examiner were tightly controlled, restricted, governed by confidentiality obligations, and conducted according to protocols that did not disclose the claimed features of the invention (see *Dey, L.P. v. Sunovion Pharmaceuticals, Inc.*). Accordingly, it is clear that the public was not in possession of the claimed sequences of eculizumab prior to the effective filing date of the present application, and for at least this reason, the Examiner is respectfully requested to reconsider and withdraw the rejection.

Rejection Under 35 U.S.C. § 103

Claims 1-10 are rejected under 35 U.S.C. § 103 as being unpatentable over Hillmen *et al.* (*N. Engl. J. Med.* 2004; 350:552-9), in view of Thomas *et al.* (Molecular Immunology, 33(17/18), pp.

1389-1401, 1996) and Evans *et al.* (US 6,355,245) and further in view of Wang (US 2005/0271660).

Applicants respectfully traverse the rejection. As discussed above, neither eculizumab nor its complete sequence were available to the public or in "public use" prior to the March 15, 2007 effective filing date of the present application. Therefore, the Examiner's underlying premise supporting the present obviousness rejection (*i.e.*, that eculizumab was publicly known or used prior to March 15, 2007) is factually incorrect. Moreover, none of Hillmen *et al.*, Thomas *et al.*, Evans *et al.* or Wang teach or suggest the complete sequence of eculizumab. Thus, the Examiner has failed to establish a prima facie case of obviousness.

Accordingly, for at the least the above-discussed reasons, the pending claims are patentable and the Examiner is respectfully requested to reconsider and withdraw this rejection.

Obviousness-Type Double Patenting

The Examiner has rejected claims 1-10 on the ground of non-statutory obviousness-type double patenting as being unpatentable over:

1. Claims 1-19 of US Patent No: 9,494,601 in view of Hillmen *et al.*, Thomas *et al.*, Evans *et al.*, and Wang;
2. Claims 1-17 of US Patent No: 9,409,980 in view of Hillmen *et al.*, Thomas *et al.*, Evans *et al.*, and Wang;
3. Claims 1-14 of US Patent No: 9,556,263 in view of Hillmen *et al.*, Thomas *et al.*, Evans *et al.*, and Wang.

While in no way acquiescing to the rejection, Applicants respectfully submit herewith a terminal disclaimer over US Patent No: 9,494,601, US Patent No: 9,409,980, and US Patent No: 9,556,263, thereby obviating the rejection. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Response to Request for Additional Information Under 37 C.F.R. § 1.105

In response to the Examiner's specific inquiries and request for additional information under 37 C.F.R. § 1.105 (Rule 105 Request), Applicants provide a copy of the (1) Amendment and Response and (2) Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone that were submitted in related U.S. Patent Application No: 15/148,839 ("the '839 application") on May 12, 2017.

The interrogatories set forth in the Rule 105 Request for the present case consist of a subset of the interrogatories set forth in the Rule 105 Request issued in the '839 application (as part of the Office Action dated April 4, 2017 ("'839 Office Action"))). Specifically, the interrogatories and corresponding Exhibits in the present case are identical to those raised in the '839 application, except that Exhibits C and F from the '839 Office Action (and the corresponding interrogatories) were not included in the Rule 105 Request for the present case.

Accordingly, in response to the Rule 105 Interrogatories (A-E) for the present case, Applicants refer the Examiner to Applicants' response to the identical Interrogatories (i.e., A, B, D, E and G) for the '839 application, provided in the enclosed Amendment and Response and Declaration submitted in the '839 application dated May 12, 2017.

CONCLUSION

If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call Applicants' attorney at (617) 217-4630. The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3.

Dated: June 13, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicants

Copy of Amendment and Response and

Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone,

Filed on May 12, 2017 in U.S. Patent Application No: 15/148,839

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: May 12, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/148,839 Confirmation No.: 3159

Filed: May 6, 2016 Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.111

Dear Sir:

In response to the Office Action dated April 4, 2017 (Paper No. 20170227), please amend the above-identified U.S. patent application as follows:

A Listing of the Claims begins on page 2 of this paper.

Remarks/Arguments begin on page 3 of this paper.

LISTING OF THE CLAIMS

1. **(Previously Presented)** A pharmaceutical composition for use in treating a patient afflicted with paroxysmal nocturnal hemoglobinuria (PNH), wherein the composition is a sterile, preservative free, 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml antibody solution, wherein the antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

2. **(Previously Presented)** A pharmaceutical composition comprising an anti-C5 antibody, wherein the anti-C5 antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

3. **(Previously Presented)** The pharmaceutical composition of claim 2, wherein the pharmaceutical composition is a sterile, preservative free 300 mg single-use dosage form comprising 30 ml of a 10 mg/ml anti-C5 antibody solution.

REMARKS

Claims 1-3 are pending.

Interview Summary

Applicants thank the Examiner and his Supervisor for the interview held on May 1, 2017 and follow-up discussion on May 5, 2017, during which the outstanding rejections and related Rule 37 C.F.R. § 1.105 Request for Additional Information in the above-referenced application were discussed. The substance of the interview and follow-up discussion is reflected in the following remarks.

Moreover, as confirmed in the Interview Summary dated May 11, 2017, if the Examiners determine after consideration of the present response and accompanying Declaration that there was no 35 U.S.C. § 102(b) "public use" of the claimed invention, then there also was no 35 U.S.C. § 102(a) "knowledge or use" of the claimed invention.

Information Disclosure Statement

Applicants acknowledge with appreciation that the Information Disclosure Statements filed on May 6, 2016 and February 15, 2017 have been considered by the Examiner. Additionally, Applicants submit herewith a further Supplemental Information Disclosure Statement to make of record additional information for the Examiner's consideration.

Rejection of Claim 2 Under 35 U.S.C. § 102(b)

The Examiner has rejected claim 2 as allegedly being anticipated by the following references, which the Examiner has characterized as follows:

Cited Reference	Examiner's Characterization
Hillmen *et al.* (*N. Engl. J. Med.* 2004; 350; 552-9), as evidenced by Applicant's specification as filed at page 44 and Thomas *et al.* (*Molecular Immunology*, 33 (17/18), pp. 1389-1401, 1996)	Hillmen *et al.* teaches a method for treating a human patient that suffers from PNH by administering to the patient an effective amount of eculizumab. Applicant's specification at page 44 discloses that SEQ ID NOs: 2 and 4 constitute the heavy and light chains of eculizumab. Thomas *et al.* teach the humanized h5G1.1 antibody, which retains the affinity and blocking functions of its murine counterpart.

Appel *et al*. (*Kidney International*, 70, S45-S50, 2006)	Appel *et al*. teaches that eculizumab is a humanized monoclonal antibody that prevents the activation of complement component C5.
Wang (US2005/0271660)	Wang teaches that eculizumab inhibits activity of the complement system.

The Examiner acknowledges that none of the cited references specifically teaches that eculizumab comprises heavy and light chains consisting of the amino acid sequences set forth SEQ ID NO:2 and SEQ ID NO:4, respectively. However, the Examiner is of the opinion that, absent evidence to the contrary, these heavy and light chain sequences are "inherent properties" of eculizumab.

Applicants respectfully traverse the rejection. As explained in the April 4, 2017 Office Action on pages 3-4, the premise of the Examiner's inherency rejection is that eculizumab (and thus, its full length sequence) "was effectively in the public domain", as evidenced by Exhibit A (studies 41-60 from clinicaltrials.gov), Exhibit B (printout for NCT00005571), Exhibit C (clinicaltrials.gov from 6/23/2005 for NCT00098280), Exhibit D (Kaplan 2002), Exhibit E (Appel *et al.* 2006), Exhibit F (Wang, US20050271660), Exhibit G (Rother, WO 2005/110481), and Exhibit H (Rother, US 2009/0028850). However, this is incorrect. Neither eculizumab nor its complete sequence, including the sequence of its unique, non-naturally occurring, protein-engineered heavy chain, was in the public domain prior to the March 15, 2007 effective filing date of the present application, as discussed in detail below. Accordingly, the presently claimed pharmaceutical composition comprising eculizumab (and the specific sequences thereof) was also not in the public domain prior to that time.

A. Exhibits A-C: Clinical Trials

With respect to the clinical trials cited by the Examiner in Appendices A-C, these clinical trials did not constitute "public use" of eculizumab under pre-AIA 35 U.S.C. § 102(b), as they were controlled, restricted, and subject to strict confidentiality obligations. Specifically, as discussed in the enclosed Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone, the cited clinical trials using eculizumab were strictly controlled and governed by clinical trial protocols which included explicit confidentiality requirements. The Federal Circuit has clearly held that clinical trials performed under such confidentiality and restrictions do not constitute

"public use" within the meaning of 35 U.S.C. § 102(b). In *Dey, L.P. v. Sunovion Pharmaceuticals, Inc.* (No. 2012-1428, Fed. Cir. May 20, 2013) the Federal Circuit considered whether a clinical trial constitutes an invalidating "public use" under 35 U.S.C. § 102(b). Sunovion, the accused infringer, asserted that its own clinical trial was an invalidating public use against Dey's patents, because they involved the accused formulation and took place before Dey's 102(b) critical date. The Federal Circuit concluded that the trial was not an invalidating public use of the study medication based on the following factors: the nature of the activity that occurred in public; the public access to and knowledge of the public use; and whether there was any confidentiality obligation imposed on persons who observed the use (citing *Bernhardt, L.L.C. v. Collezione Europa USA, Inc.*, 386 F.3d 1371, 1379 (Fed. Cir. 2004)). Applying this standard, the Federal Circuit reversed the district court's grant of summary judgment to Sunovion because the clinical trial's restrictions, instructions, and records requirements supported a conclusion ***that the trial was sufficiently controlled and restricted and therefore, not an invalidating public use***. In addition, the Court noted that the ***investigators (i.e., "the most knowledgeable persons involved in the study") were under express confidentiality restrictions, and neither the patients nor the doctors were aware of the claimed particulars of Sunovion's formulation*** (page 10 of *Dey*). Specifically, "while it is true that participants were permitted to discuss the study with their doctors, ***they were not in a position to reveal the composition…because they were unaware of the specifics of the inventive formulations***" (page 10 of *Dey*). As argued by Dey and agreed by the Court, "***if members of the public are not informed of, and cannot readily discern, the claimed features of the invention in the allegedly invalidating prior art, the public has not been put in possession of those features***."

Similar to the facts in *Dey*, the trials cited in Exhibits A-C on page 3 of the Office Action were tightly controlled, restricted and governed by express confidentiality provisions, as discussed in detail in the enclosed Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone. Moreover, similar to the facts in *Dey*, the clinical trial protocols did not contain the specifics of the claimed invention or allow the participants to readily discern the features of the presently claimed invention, including the sequence of the antibody being administered, *i.e.*, eculizumab. Thus, in accordance with *Dey*, the cited clinical trials did not constitute "public use" of eculizumab under pre-AIA 35 U.S.C. § 102(b).

B. Exhibits D-H: Publications

With respect to the publications cited in Exhibit D (Kaplan 2002), Exhibit E (Appel *et al.* 2006), Exhibit F (Wang, US20050271660), Exhibit G (Rother, WO 2005/110481), and Exhibit H (Rother, US 2009/0028850), the Examiner is of the opinion that these publications demonstrate that "eculizumab was effectively in the public domain" prior to the effective filing date of the present application. In support of this assertion, the Examiner cites *In re Antor Media Corp* (CAFC, 2012) for the proposition that "the subject matter disclosed in a reference, whether it is a ***patent claim***, ***other parts of a patent***, or non-patent literature, is presumed to be enabling absent evidence to the contrary (emphasis added)."

Applicants respectfully disagree with the Examiner's position. From the outset, as noted by the Court in *In re Antor Media Corp*, the question of enablement only arises if the cited reference in fact teaches each and every element of the claimed invention (page 9 of *In re Antor Media Corp*). In the present case, none of the publications cited in Exhibits D-H teach the complete structure of eculizumab, as presently claimed, including its complete sequence with a unique, non-naturally occurring, protein-engineered heavy chain. Nor do these references enable the presently claimed full biochemical structure of eculizumab, for the reasons discussed above. Exhibit D (Kaplan 2002) describes clinical studies that used eculizumab. Exhibit E (Appel *et al.* 2006) merely discloses that eculizumab is a humanized monoclonal antibody that prevents the activation of complement component C5. Exhibit F (Wang, US20050271660) discloses antibody formulations suitable for nebulization using murine antibodies in the examples. Exhibit G (Rother, WO 2005/110481) and Exhibit H (Rother, US 2009/0028850, which was not published until after March 16, 2007) disclose the use of eculizumab for treating PNH patients, as well as the use of the mouse anti-C5 antibody BB5.1 in prolonging the survival of an allograft. Importantly, none of the cited references teach the sequence of eculizumab, let alone its unique, non-naturally occurring, protein-engineered full heavy chain (including the CH1-hinge-CH2-CH3 regions).

As discussed above, to anticipate a claimed invention, a reference must provide an enabling disclosure of the desired subject matter. Specifically, pursuant to M.P.E.P § 2121.1, "***mere naming or description of the subject matter is insufficient,*** if it cannot be produced without undue experimentation. *Elan Pharm., Inc. v. Mayo Found. For Med. Educ. & Research,*

346 F.3d 1051, 1054, 68 USPQ2d 1373, 1376 (Fed. Cir. 2003) (emphasis added)." Further, a "reference contains an 'enabling disclosure' if the public was in possession of the claimed invention before the date of invention." Such "***possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his [or her] own knowledge to make the claimed invention***. *In re Donohue,* 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985) (emphasis added)."

It is clear that the public was not in possession of the claimed sequences of eculizumab prior to the effective filing date of the present application by virtue of the cited publications, because the ***mere naming of eculizumab is insufficient to enable*** the claimed sequences and ***one of ordinary skill in the art could not have readily determined the sequences based on the limited disclosure in the publications and his or her own general knowledge***.

C. Conclusion

In sum, none of the cited references teach or enable the complete sequence of eculizumab, as presently claimed. Moreover, eculizumab was not in "public use" under 35 U.S.C. § 102(b) prior to the March 15, 2007 effective filing date of the present application, at least because the clinical trials cited by the Examiner were tightly controlled, restricted, governed by confidentiality obligations, and conducted according to protocols that did not disclose the claimed features of the invention (see *Dey, L.P. v. Sunovion Pharmaceuticals, Inc.*). Accordingly, it is clear that the public was not in possession of the claimed sequences of eculizumab prior to the effective filing date of the present application, and the Examiner is respectfully requested to reconsider and withdraw the rejection.

Rejection of Claims 1-3 Under 35 U.S.C. § 103(a)

Claims 1-3 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hillmen *et al.* (*N. Engl. J. Med.* 2004; 350; 552-9), in view of Evans *et al.* (US 6,355,245) and Wang (2005/0271660), and as evidenced by the parent specification (US Patent Application No.: 13/426,973). The Examiner asserts that Applicants' argument that the complete structure of eculizumab was not disclosed in the art or available to the public is unpersuasive because "the preponderance of evidence indicates that the antibody was in public use" and this public

availability was "sufficient to have placed one of skill in the art in possession of applicant's currently claimed sequences of eculizumab."

Applicants respectfully traverse the rejection. As discussed above, neither eculizumab nor its complete sequence were available to the public or in "public use" prior to the March 15, 2007 effective filing date of the present application. Therefore, the Examiner's underlying premise supporting the present obviousness rejection (*i.e.*, that eculizumab was in "public use") is factually incorrect. Moreover, as acknowledged by the Examiner on page 7 of the Office Action, none of Hillmen *et al.*, Evans *et al.* or Wang teach or suggest the complete sequence of eculizumab. Clearly, the Examiner has failed even to establish a prima facie case of obviousness.

Accordingly, for at the least the above-discussed reasons, the pending claims are patentable and the Examiner is respectfully requested to reconsider and withdraw this rejection.

Obviousness-Type Double Patenting

The Examiner has rejected claims 1-3 on the ground of non-statutory obviousness-type double patenting as being unpatentable over:

1. Claims 1-9 of US Patent No: 9,494,601 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
2. Claims 1-18 of US Patent No: 8,999,340 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
3. Claims 1-17 of US Patent No: 9,409,980 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
4. Claims 3-14 of US Patent No: 9,556,263 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
5. Claims 1-22 of US Patent No: 9,352,035 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
6. Claims 1-14 of US Patent No: 9,415,102 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
7. Claims 1-17 of US Patent No: 9,409,980 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
8. Claims 1-4 of US Patent No: 9,447,176 in view of Hillmen *et al.*, Evans *et al.*, and Wang;

9. Claims 1-5, 7, 9-16 of US Patent No: 9,388,235 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
10. Claims 15-17 and 58-59 of US Patent Application No: 11/127,438 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
11. Claims 1-4 of US Patent Application No: 15/243,386 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
12. Claims 1-4 of US Patent Application No: 15/243,290 in view of Hillmen *et al.*, Evans *et al.*, and Wang;
13. Claims 94 and 97-98 of US Patent Application No: 15/282,464 in view of Hillmen *et al.*, Evans *et al.*, and Wang; and
14. Claims 129-138 of US Patent Application No: 15/148,491 in view of Hillmen *et al.*, Evans *et al.*, and Wang.

While in no way acquiescing to the rejection, Applicants respectfully submit herewith a terminal disclaimer over US Patent No: 9,494,601, US Patent No: 8,999,340**,** US Patent No: 9,409,980,US Patent No: 9,556,263, US Patent No: 9,352,035, US Patent No: 9,415,102, US Patent No: 9,447,176, US Patent No: 9,388,235, US Patent Application No: 15/243,290, US Patent Application No: 15/282,464, and US Patent Application No: 15/148,491.

With respect to US Patent Application No: 15/243,386, Applicants note that this application lapsed on April 20, 2017 for failure to respond to the Office Action dated October 19, 2016. Accordingly, the rejection relating to the '386 application has been rendered moot.

Finally, with respect to US Patent Application No: 11/127,438, while the Examiner acknowledges that "the conflicting claims are not identical", the Examiner asserts that "they are not patentably distinct from each other because both the current application and the '438 claim [application] claim eculizumab." Applicants respectfully traverse this rejection.

The pending claims are drawn to pharmaceutical compositions comprising *eculizumab as defined by the complete heavy and light chain sequences of the antibody*. In contrast, claims 15-17 and 58-59 of the '438 application are drawn to an *aerosol composition* comprising h5G1.1, which is useful for the treatment of asthma. Importantly, the claims of the '438 application fail to recite any part of the sequence of eculizumab, let alone the complete heavy and light chain sequences. Nor does the specification of the '438 application teach, enable or in any way render

Samsung Bioepis Exhibit No. 1002
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obvious the complete structure of eculizumab, as presently claimed. Moreover, as discussed above and in the attached Declaration, neither eculizumab nor its complete sequence were available to the public or in "public use" prior to the March 15, 2007 effective filing date of the present application. Accordingly, the mere naming of h5G1.1 in the claims of the '438 application does not render the present claims anticipated by or obvious over those of the '438 application.

A "nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s)" (see MPEP § 804). In view of the above, it is clear that the pending claims of the present '839 application and the pending claims of the '438 application are ***patentably distinct***. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Response to Request for Additional Information Under 37 C.F.R. § 1.105

In response to the Examiner's specific inquiries and request for additional information under 37 C.F.R. § 1.105, Applicants provide the following factual information, as well as the enclosed Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone.

(1) Interrogatories A–F

As attested in the enclosed Declaration, Exhibits A through F cited by the Examiner on pages 7-9 of the Office Action reference 17 different clinical studies using eculizumab that occurred prior to March 16, 2007. Of the 17 studies, 4 are referred to as "extension" studies, which are studies that are appended to a clinical study to prolong the treatment and/or monitoring period of the same patients that participated in the initial study.

Of these 17 clinical studies, one that is described in both Exhibits D and F and entitled, "A Randomized, Third-Party-Blind, Placebo-Controlled, Pilot Study of the Effect of h5G1.1-mAb in Patients with Bullous Pemphigoid," only enrolled one patient, and that one patient was randomized to receive placebo. Thus, no patient received eculizumab in the course of this study and the study was terminated.

For the remaining 16 clinical studies identified in Exhibits A through F, the below table includes: the full name of each study (and extension study thereof, if applicable, identified by an "E" or an "X" in the study number), the study number(s), the Exhibit(s) cited by the Examiner on pages 7-8 of the Office Action that each study was described in, and whether the study (including any extension study thereof) included a confidentiality provision (as described in further detail below).

Study Name[1]	**Study Number**	**Exhibit(s)**	**Explicit Confidentiality Provision**
An Open-Label Study of the Effect of Eculizumab (h5G1.1-mAb) on Patients with Transfusion Dependent, Hemolytic, Paroxysmal Nocturnal Hemoglobinuria	C02-001	F	Yes
Extension Study of Eculizumab in Patients with Transfusion Dependent, Haemolytic, Paroxysmal Nocturnal Haemoglobinuria Who Previously Participated in Study C02-001	E02-001		
An Open-Label Extension Study of Eculizumab in Patients with Transfusion Dependent, Haemolytic, Paroxysmal Nocturnal Hemoglobinuria Who Previously Participated in Study E02-001	X03-001		
A Hemoglobin Stabilization and Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria Patients (TRIUMPH)	C04-001	A[2], F	Yes
Safety in Hemolytic PNH Patients Treated with Eculizumab: A Multi-Center Open-Label Research Design Study (SHEPHERD)	C04-002	A, C, F	Yes

[1] Although some of these studies may not have occurred in the United States and thus are not available to give rise to public use under 35 U.S.C. § 102(b), all of the trials have been in included in this table (regardless of where they occurred).

[2] Of the studies referenced in Exhibit A, studies 41-43, 45-46, 52-54, 56-58 and 60 are all indicated as having been "received" or "started" *after* March 16, 2007, *i.e.*, the date eculizumab was first approved for commercial marketing and sale. Thus, only studies identified as numbers 44, 47-51, 55 and 59 in Exhibit A are included in this table.

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Study Name[1]	Study Number	Exhibit(s)	Explicit Confidentiality Provision
A Phase III, Open-Label, Extension Study of Eculizumab in Patients with Transfusion Dependent, Hemolytic Paroxysmal Nocturnal Hemoglobinuria (PNH) Who Have Participated in the TRIUMPH (C04-001), SHEPHERD (C04-002) or X03-001 Studies	E05-001	A, F	Yes
A Randomized, Double-Blind, Placebo-Controlled Pilot Study of the Effect of h5G1.1-mAb on Dermatomyositis Patients	C99-006	B, D[3], F	Yes
Phase I Study of h5G1.1-mAb in Patients with Rheumatoid Arthritis	C97-001	F	Yes
A Phase II Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on Rheumatoid Arthritis Patients Receiving Methotrexate	C99-001	D, F	Yes
A Phase IIB Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on Rheumatoid Arthritis (RA) Patients with Active Disease Despite Receiving Methotrexate or Leflunomide	C01-004	D, F	Yes
A Phase II Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on the Reduction of Proteinuria in Patients with Idiopathic Membranous Glomerulopathy An Open Label Study of the Long-Term Safety of h5G1.1-mAb on the Reduction of Proteinuria in Patients with Idiopathic Membranous Glomerulopathy	C99-004 E99-004	D, E, F	Yes
Phase I Study of h5G1.1-mAb in Patients with Systemic Lupus Erythematosus	C97-002	D, F	Yes
A Randomized, Double-Blind, Placebo-Controlled Pilot Study of the Effect of h5G1.1-mAb on Psoriatic Patients	C99-007	D, F	Yes
The Paroxysmal Nocturnal Hemoglobinuria Early Access Treatment Protocol (EMBRACE)	C06-002	F	Yes
PNH Registry	M07-001	A, F	Yes

[3] Item 445382 referenced in the Examiner's question regarding the Kaplan reference (Exhibit D) does not refer to an actual study. However, to address any potential questions the Examiner has with respect to Exhibit D, the Kaplan reference has been reviewed in its entirety and the clinical studies described therein that used eculizumab have been included.

(i) The Antibody Sequences Used in the Clinical Studies

As attested in the enclosed Declaration, the antibody (eculizumab) used in each of the studies included in the above table contained the heavy and light chain sequences of SEQ ID NOs: 2 and 4. However, the protocols used to conduct those studies did not inform either doctors or patients of the sequences.

(ii, iii) Confidentiality Provisions in the Clinical Studies

As indicated in the table above and discussed in the enclosed Declaration, in the course of conducting each of the clinical trials listed, participating physicians and others involved in the study were required to maintain Alexion's confidential and/or proprietary information in strict confidence. For example, these individuals were required to maintain the confidentiality of the clinical trial protocols themselves, as each protocol was marked "Confidential". These individuals were also bound by explicit confidentiality provisions in the protocol. As attested to in the attached Declaration of Dr. Boone, the following language from the protocol for study number C04-001, which refers to the Phase III TRIUMPH study, provides a representative example of the confidentiality requirements set forth directly within these clinical study protocols:

> "THE INVESTIGATOR AND ALL EMPLOYEES AND COWORKERS INVOLVED WITH THIS STUDY SHALL NOT DISCLOSE OR USE FOR ANY PURPOSE, OTHER THAN PERFORMANCE OF THE STUDY, ANY DATA, RECORDS OR OTHER UNPUBLISHED, CONFIDENTIAL INFORMATION DISCLOSED TO THOSE INDIVIDUALS FOR THE PURPOSE OF THE STUDY. PRIOR WRITTEN AGREEMENT FROM THE SPONSOR OR ITS DESIGNEE MUST BE OBTAINED FOR THE DISCLOSURE OF ANY SAID CONFIDENTIAL INFORMATION TO OTHER PARTIES."

As another representative example, Dr. Boone cites the protocol for the study in dermatomyositis (study number C02-002), which stated:

> "THIS CONFIDENTIAL INFORMATION ABOUT AN INVESTIGATIONAL DRUG IS PROVIDED FOR THE EXCLUSIVE USE OF INVESTIGATORS

> OF THIS DRUG AND IS SUBJECT TO RECALL AT ANY TIME. THE INFORMATION IN THIS DOCUMENT MAY NOT BE DISCLOSED UNLESS SUCH DISCLOSURE IS REQUIRED BY FEDERAL OR STATE LAW OR REGULATIONS SUBJECT TO THE FOREGOING. THIS INFORMATION MAY BE DISCLOSED ONLY TO THOSE PERSONS INVOLVED IN THE STUDY WHO HAVE A NEED TO KNOW WITH THE OBLIGATION NOT TO FURTHER DISSEMINATE THIS INFORMATION. THESE RESTRICTIONS ON DISCLOSURE WILL APPLY EQUALLY TO ALL FUTURE ORAL OR WRITTEN INFORMATION SUPPLIED TO YOU BY ALEXION, WHICH IS DESIGNATED AS 'PRIVILEGED' OR 'CONFIDENTIAL'."

As Dr. Boone explains, all confidentiality provisions in the protocols for the studies listed in the table above were substantively identical to these representative confidentiality provisions from studies C04-001 and C02-002.

In addition, Dr. Boone attests that each clinical trial protocol required strict control over the physical drug product (eculizumab). For example, the clinical trial protocol for the TRIUMPH study described the secure storage and inventory and disposal requirements of each participating physician and study site as shown below:

> "Eculizumab must be stored in a secure, limited-access storage area. . . .
>
> Test Article Accountability…When a drug shipment is received at the site, the pharmacist at each site shall verify the contents, sign the packing invoice provided with the shipment, and maintain the original copy for review by the study monitor. The duplicate copy should be kept in the pharmacy binder.
>
> Each shipment of investigational drug supplies will contain an accountability log to assist the pharmacist in maintaining current and accurate inventory records covering receipt, dispensing, and disposition of the investigational product. During the study, the following information must be noted in the accountability log: the identification number(s), initials of patient(s) who is dispensed investigational product, the date(s) that the investigational product is prepared and dispensed, and the initials of the pharmacist or designee who prepared the investigational product. Empty vials and vials with residual materials should be kept for inspection by the study monitor.
>
> The study monitor will examine the inventory records during study conduct. Additionally, the inventory records must be readily available and may be subject

> to regulatory authorities or an independent auditor's inspection at any time. At the completion of the study, to satisfy regulatory requirements regarding drug accountability, all remaining investigational product inventory will be reconciled and retained or destroyed according to applicable local, state, and federal regulations."

Dr. Boone also explains that all of the protocols for the studies listed in the above table included similar (if not identical) drug storage and accountability provisions, except for the PNH Registry protocol (the last entry in the table above). The PNH Registry was not a study in which eculizumab was administered to any patients; rather, it was a "non-interventional study designed to collect post-marketing pharmacovigilance and effectiveness data on patients treated with Soliris® (eculizumab)" and to "collect information on the progression of disease in the Soliris treated and untreated PNH patient population." Accordingly, the protocol for the PNH Registry did not contain drug storage or accountability provisions. The PNH Registry protocol did, however, include strict confidentiality provisions as indicated in the table above, *e.g.*, requiring all physicians to "agree to maintain the confidentiality of all information received or developed in connection with [the] protocol."

(iv) Physicians Acting at Alexion's Direction

As discussed in the enclosed Declaration, to participate in each clinical trial listed in the table above, each physician had to sign the protocol for the trial, committing to adhere to the respective clinical trial protocol's provisions. For example, the TRIUMPH study protocol, just prior to the physician's signature line, stated:

> "I agree to conduct the study as outlined in the protocol entitled: *"TRIUMPH*: A Hemoglobin Stabilization and Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria Patients" and in accordance with the guidelines and all applicable government regulations. I have read and understand all sections of the protocol including Section 11, Administrative Considerations."

As Dr. Boone states, all of the other protocols similarly required each physician (or investigator) to agree to conduct the study in accordance with the protocol.

(2) Interrogatory G

As attested in the enclosed Declaration, the antibody having the heavy chain described by SEQ ID NO:2 and the light chain described by SEQ ID NO:4 was first approved for commercial marketing and sale on March 16, 2007.

CONCLUSION

In view of the above amendment, arguments, and accompanying Declaration, Applicants believe the pending application is in condition for allowance. If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call Applicants' attorney at (617) 217-4630.

Applicants believe no fee is required with this submission. However, should any additional fee be due, the Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN2.

Dated: May 12, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicant

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: May 12, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/148,839

Confirmation No.: 3159

Filed: May 6, 2016

Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 C.F.R. § 1.132 BY DR. LAURAL BOONE

I, Laural S. Boone, J.D., Ph.D., declare the following:

1. I am presently a Senior Director and Patent Counsel at Alexion Pharmaceuticals, Inc., (hereafter, "Alexion") which is located in New Haven, Connecticut. I have been employed in this capacity at Alexion for three years to date. Alexion is the Assignee of the above-referenced patent application. I received a B.A. in Chemistry from Barnard College, a M.S. and Ph.D. in Organic Chemistry from the University of Chicago, and a J.D. from the George Washington University Law School. I also completed a Postdoctoral Fellowship in Cellular and Molecular Biophysics at Rockefeller University. I am registered to practice as a patent attorney before the United States Patent & Trademark Office.

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2. I have reviewed the Examiner's request for additional information under 37 C.F.R. § 1.105 and provide the following statements.

3. I have reviewed Exhibits A through F cited by the Examiner on pages 7-8 of the April 4, 2017 Office Action, including each of the 37 documents listed in Exhibit F. These exhibits and the documents listed therein reference 17 different clinical studies using eculizumab that occurred prior to March 16, 2007. Of the 17 studies, 4 are referred to as "extension" studies, which are studies that are appended to a clinical study to prolong the treatment and/or monitoring period of the same patients that participated in the initial study.

4. Of these 17 clinical studies, one that is described in both Exhibits D and F, is entitled "A Randomized, Third-Party-Blind, Placebo-Controlled, Pilot Study of the Effect of h5G1.1-mAb in Patients with Bullous Pemphigoid." Based on my review of communications between Alexion employees and physicians participating in this study, I have determined that only one patient was enrolled in this study, and that the one patient was randomized to receive placebo. Thus, no patient received eculizumab in the course of this study, and the study was terminated.

5. For the remaining 16 clinical studies identified in Exhibits A through F, I have reviewed each of the clinical trial protocols and the following information from the protocols is shown in the table below: the full name of each study (and extension study thereof, if applicable, identified by an "E" or an "X" in the study number), the study number(s), the Exhibit(s) cited by the Examiner on pages 7-8 of the Office Action that each study was described in, and whether the study (including any extension study thereof) included a confidentiality provision (as described in further detail below).

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Study Name[1]	Study Number	Exhibit(s)	Explicit Confidentiality Provision
An Open-Label Study of the Effect of Eculizumab (h5G1.1-mAb) on Patients with Transfusion Dependent, Hemolytic, Paroxysmal Nocturnal Hemoglobinuria Extension Study of Eculizumab in Patients with Transfusion Dependent, Haemolytic, Paroxysmal Nocturnal Haemoglobinuria Who Previously Participated in Study C02-001 An Open-Label Extension Study of Eculizumab in Patients with Transfusion Dependent, Haemolytic, Paroxysmal Nocturnal Hemoglobinuria Who Previously Participated in Study E02-001	C02-001 E02-001 X03-001	F	Yes
A Hemoglobin Stabilization and Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria Patients (TRIUMPH)	C04-001	A[2], F	Yes
Safety in Hemolytic PNH Patients Treated with Eculizumab: A Multi-Center Open-Label Research Design Study (SHEPHERD)	C04-002	A, C, F	Yes
A Phase III, Open-Label, Extension Study of Eculizumab in Patients with Transfusion Dependent, Hemolytic Paroxysmal Nocturnal Hemoglobinuria (PNH) Who Have Participated in the TRIUMPH (C04-001), SHEPHERD (C04-002) or X03-001 Studies	E05-001	A, F	Yes
A Randomized, Double-Blind, Placebo-Controlled Pilot Study of the Effect of h5G1.1-mAb on Dermatomyositis Patients	C99-006	B, D[3], F	Yes

[1] Although some of these studies may not have occurred in the United States and thus are not available to give rise to public use under 35 U.S.C. § 102(b), all of the trials are included in this table (regardless of where they occurred).

[2] Of the studies referenced in Exhibit A, studies 41-43, 45-46, 52-54, 56-58 and 60 are all indicated as having been "received" or "started" *after* March 16, 2007, *i.e.*, the date eculizumab was first approved for commercial marketing and sale. Thus, only studies started before the marketing approval, identified as numbers 44, 47-51, 55, and 59 in Exhibit A, are included in this table.

[3] Item 445382 referenced in the Examiner's question regarding the Kaplan reference (Exhibit D) does not refer to an actual study. However, to address any potential questions the Examiner has with respect to

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Study Name[1]	Study Number	Exhibit(s)	Explicit Confidentiality Provision
Phase I Study of h5G1.1-mAb in Patients with Rheumatoid Arthritis	C97-001	F	Yes
A Phase II Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on Rheumatoid Arthritis Patients Receiving Methotrexate	C99-001	D, F	Yes
A Phase IIB Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on Rheumatoid Arthritis (RA) Patients with Active Disease Despite Receiving Methotrexate or Leflunomide	C01-004	D, F	Yes
A Phase II Randomized, Double-Blind, Placebo-Controlled Study of the Effect of h5G1.1-mAb on the Reduction of Proteinuria in Patients with Idiopathic Membranous Glomerulopathy An Open Label Study of the Long-Term Safety of h5G1.1-mAb on the Reduction of Proteinuria in Patients with Idiopathic Membranous Glomerulopathy	C99-004 E99-004	D, E, F	Yes
Phase I Study of h5G1.1-mAb in Patients with Systemic Lupus Erythematosus	C97-002	D, F	Yes
A Randomized, Double-Blind, Placebo-Controlled Pilot Study of the Effect of h5G1.1-mAb on Psoriatic Patients	C99-007	D, F	Yes
The Paroxysmal Nocturnal Hemoglobinuria Early Access Treatment Protocol (EMBRACE)	C06-002	F	Yes
PNH Registry	M07-001	A, F	Yes

Exhibit D, I reviewed the Kaplan reference in its entirety, and the clinical studies described therein that used eculizumab are also included in this table.

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6. I have reviewed an alignment of the sequence of eculizumab that is listed in the Biologic License Application for Soliris®(eculizumab) ("BLA") with the heavy and light chain sequences of SEQ ID NOs: 2 and 4 of the parent of the pending application, PCT/US2007/006606 (published as WO 2007/106585). I have concluded that the antibody (eculizumab) used in each of the studies included in the above table contained the heavy and light chain sequences of SEQ ID NOs: 2 and 4. However, the protocols used to conduct those studies did not inform either doctors or patients of the sequences.

7. As indicated in the table above, in the course of conducting each of the clinical trials listed, participating physicians and other participants in the study were required to maintain Alexion's confidential and/or proprietary information in strict confidence. For example, these individuals were required to maintain the confidentiality of the clinical trial protocols themselves, as each protocol was marked "Confidential." These individuals were also bound by explicit confidentiality provisions in the protocol. The following language from the protocol for study number C04-001, which refers to the Phase III TRIUMPH study, provides a representative example of the confidentiality requirements set forth directly within these clinical study protocols (emphasis added):

> "THE INVESTIGATOR AND ALL EMPLOYEES AND COWORKERS INVOLVED WITH THIS STUDY SHALL NOT DISCLOSE OR USE FOR ANY PURPOSE, OTHER THAN PERFORMANCE OF THE STUDY, ANY DATA, RECORDS OR OTHER UNPUBLISHED, CONFIDENTIAL INFORMATION DISCLOSED TO THOSE INDIVIDUALS FOR THE PURPOSE OF THE STUDY. PRIOR WRITTEN AGREEMENT FROM THE SPONSOR OR ITS DESIGNEE MUST BE OBTAINED FOR THE DISCLOSURE OF ANY SAID CONFIDENTIAL INFORMATION TO OTHER PARTIES."

8. As another representative example, the protocol for the study in dermatomyositis (study number C02-002) stated:

> "THIS CONFIDENTIAL INFORMATION ABOUT AN INVESTIGATIONAL DRUG IS PROVIDED FOR THE EXCLUSIVE USE OF INVESTIGATORS OF THIS DRUG AND IS SUBJECT TO RECALL AT ANY TIME. THE INFORMATION IN THIS DOCUMENT MAY NOT BE DISCLOSED UNLESS SUCH DISCLOSURE IS REQUIRED BY FEDERAL OR STATE LAW OR REGULATIONS SUBJECT TO THE FOREGOING. THIS INFORMATION MAY BE DISCLOSED ONLY TO THOSE PERSONS INVOLVED IN THE STUDY WHO HAVE A NEED TO KNOW WITH THE OBLIGATION NOT TO FURTHER DISSEMINATE THIS INFORMATION. THESE RESTRICTIONS ON DISCLOSURE WILL APPLY EQUALLY TO ALL FUTURE ORAL OR WRITTEN INFORMATION SUPPLIED TO YOU BY ALEXION, WHICH IS DESIGNATED AS 'PRIVILEGED' OR 'CONFIDENTIAL'."

9. All confidentiality provisions in the protocols for the studies listed in the table above were substantively identical to these representative confidentiality provisions from studies C04-001 and C02-002.

10. In addition, each clinical trial protocol that I reviewed also required strict control over the physical drug product (eculizumab). For example, the clinical trial protocol for the TRIUMPH study described the secure storage and inventory and disposal requirements of each participating physician and study site as shown below:

> "Eculizumab must be stored in a secure, limited-access storage area. . . .
>
> Test Article Accountability…When a drug shipment is received at the site, the pharmacist at each site shall verify the contents, sign the packing invoice provided with the shipment, and maintain the original copy for review by the study monitor. The duplicate copy should be kept in the pharmacy binder.
>
> Each shipment of investigational drug supplies will contain an accountability log to assist the pharmacist in maintaining current and accurate inventory records covering receipt, dispensing, and disposition of the investigational product. During the study, the following information must be noted in the accountability log: the identification number(s), initials of patient(s) who is dispensed investigational product, the date(s) that the investigational product is prepared and dispensed, and the initials of the pharmacist or designee who prepared the investigational product. Empty vials and vials with residual materials should be kept for inspection by the study monitor.

> The study monitor will examine the inventory records during study conduct. Additionally, the inventory records must be readily available and may be subject to regulatory authorities or an independent auditor's inspection at any time. At the completion of the study, to satisfy regulatory requirements regarding drug accountability, all remaining investigational product inventory will be reconciled and retained or destroyed according to applicable local, state, and federal regulations."

11. All of the protocols that I reviewed for the studies listed in the above table included similar (if not identical) drug storage and accountability provisions, except for the PNH Registry protocol (the last entry in the table above). The PNH Registry was not a study in which eculizumab was administered to any patients, but was a registry for all participating patients with PNH either treated or untreated. More particularly, it was a "non-interventional study designed to collect post-marketing pharmacovigilance and effectiveness data on patients treated with Soliris® (eculizumab)" and to "collect information on the progression of disease in the Soliris treated and untreated PNH patient population." Accordingly, the protocol for the PNH Registry did not contain drug storage or accountability provisions. The PNH Registry protocol did, however, include strict confidentiality provisions as indicated in the table above, *e.g.*, requiring all physicians to "agree to maintain the confidentiality of all information received or developed in connection with [the] protocol."

12. To participate in each clinical trial listed in the table above, each physician had to sign the protocol for the trial, committing to adhere to the respective clinical trial protocol's provisions. For example, the TRIUMPH study protocol, just prior to the physician's signature line, states:

> "I agree to conduct the study as outlined in the protocol entitled: *"TRIUMPH*: A Hemoglobin Stabilization and Transfusion Reduction Efficacy and Safety Clinical Investigation, Randomized, Multi-Center, Double-Blind, Placebo-Controlled, Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria Patients" and in accordance with the guidelines and all applicable government regulations. I have read and understand all sections of the protocol including Section 11, Administrative Considerations."

13. All of the other protocols that I reviewed similarly required each physician (or investigator) to agree to conduct the study in accordance with the protocol.

14. In response to the Examiner's Request (G), based on the BLA approval letter that I reviewed from the Food and Drug Administration Department of Health & Human Services to Alexion regarding eculizumab, the antibody having the heavy chain described by SEQ ID NO: 2 and the light chain described by SEQ ID NO: 4 was first approved for commercial marketing and sale on March 16, 2007.

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 Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or of any patent issued thereon.

Laural S. Boone, J.D., Ph.D. 11 MAY 2017
Date

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

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Alexion Pharmaceuticals, Inc.

Application No./Patent No.: 15/260,888 Filed/Issue Date: September 9, 2016

Titled: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Alexion Pharmaceuticals, Inc. (Name of Assignee) , a Corporation (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

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

1. [X] The assignee of the entire right, title, and interest.

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

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

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

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

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

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

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

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

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

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

1. From: Leonard Bell To: Alexion Pharmaceuticals, Inc.
   The document was recorded in the United States Patent and Trademark Office at
   Reel 039718, Frame 0748, or for which a copy thereof is attached.

2. From: Russell P. Rother To: Alexion Pharmaceuticals, Inc.
   The document was recorded in the United States Patent and Trademark Office at
   Reel 039718, Frame 0748, or for which a copy thereof is attached.

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

STATEMENT UNDER 37 CFR 3.73(c)

3. From: Mark J. Evans To: Alexion Pharmaceuticals, Inc.
The document was recorded in the United States Patent and Trademark Office at
Reel 041065 , Frame 0336 , or for which a copy thereof is attached.

4. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

5. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

6. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

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

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

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

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

Signature | Date: June 13 2017

Todd Spalding
Printed or Typed Name

Vice President, Head of Intellectual Property
Title or Registration Number

PTO/SB/17 (03-13)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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FEE TRANSMITTAL

		Complete if known	
		Application Number	15/260,888-Conf. #2558
		Filing Date	September 9, 2016
☐	Applicant asserts small entity status. See 37 CFR 1.27.	First Named Inventor	Leonard BELL
☐	Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.	Examiner Name	J. L. Rogers
		Art Unit	1644
TOTAL AMOUNT OF PAYMENT	($) 340.00	Practitioner Docket No.	AXJ-114BUSCN3

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☒ Deposit Account Deposit Account Number: 12-0080 Deposit Account Name: Nelson Mullins Riley & Scarborough LLP

For the above-identified deposit account, the Director is hereby authorized to (check all that apply):

☒ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☒ Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17 ☒ Credit any overpayment of fee(s)

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.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES (U = undiscounted fee; S = small entity fee; M = micro entity fee)

	FILING FEES			SEARCH FEES			EXAMINATION FEES			
Application Type	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**Fees Paid ($)**
Utility	280	140*	70	600	300	150	720	360	180	
Design	180	90	45	120	60	30	460	230	115	
Plant	180	90	45	380	190	95	580	290	145	
Reissue	280	140	70	600	300	150	2,160	1,080	540	
Provisional	260	130	65	0	0	0	0	0	0	

* The $140 small entity status filing fee for a utility application is further reduced to $70 for a small entity status applicant who files the application via EFS-Web.

2. EXCESS CLAIM FEES

Fee Description	**Undiscounted Fee ($)**	**Small Entity Fee ($)**	**Micro Entity Fee ($)**
Each claim over 20 (including Reissues)	80	40	20
Each independent claim over 3 (including Reissues)	420	210	105
Multiple dependent claims	780	390	195

Total Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**	**Multiple Dependent Claims**	
				Fee ($)	**Fee Paid ($)**
_____ - 20 or HP =	_____ x	_____ =	_____	_____	_____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
_____ - 3 or HP =	_____ x	_____ =	_____

HP = highest number of independent claims paid for, if greater than 3.

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $400 ($200 for small entity) ($100 for micro entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	**Extra Sheets**	**Number of each additional 50 or fraction thereof**	**Fee ($)**	**Fee Paid ($)**
_____ - 100 =	_____ /50 =	_____ (round **up** to a whole number) x	_____ =	_____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English specification, $130 fee (no small or micro entity discount)	
Non-electronic filing fee under 37 CFR 1.16(t) for a utility application, $400 fee ($200 small or micro entity)	
Other (e.g., late filing surcharge): 1806 Submission of an Information Disclosure Statement	180.00
1814 Statutory disclaimer, including terminal ...	160.00

SUBMITTED BY

Signature	/Jill Gorny Sloper, Esq./	Registration No. (Attorney/Agent)	60,760	Telephone	(617) 217-4630
Name (Print/Type)	Jill Gorny Sloper, Esq.			Date	June 13, 2017

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: June 13, 2017 Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Electronic Acknowledgement Receipt

EFS ID:	29486469
Application Number:	15260888
International Application Number:	
Confirmation Number:	2558
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Carolyn Blake
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3
Receipt Date:	13-JUN-2017
Filing Date:	09-SEP-2016
Time Stamp:	18:03:22
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	Fee Worksheet (SB06)	AXJ-114BUSCN3_Fee-Transmittal.pdf	32380 a705aa76c4bfd5fc446a29c660c6daebb4bb7cb6	no	1
Warnings:					

Information:	
Total Files Size (in bytes):	32380

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

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

PTO/AIA/26 (04-14)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	Docket Number (Optional) AXJ-114BUSCN3

In re Application of: Leonard BELL *et al.*

Application No.: 15/260,888-Conf. #2558

Filed: September 9, 2016

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

The applicant, Alexion Pharmaceuticals, Inc., owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of **prior patent** No. (see attached) as the term of said **prior patent** is presently shortened by any terminal disclaimer. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the **prior patent** are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the **prior patent**, "as the term of said **prior patent** is presently shortened by any terminal disclaimer," in the event that said **prior patent** later:

expires for failure to pay a maintenance fee;
is held unenforceable;
is found invalid by a court of competent jurisdiction;
is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
has all claims canceled by a reexamination certificate;
is reissued; or
is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. [X] The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

2. [ ] The undersigned is an attorney or agent of record. Reg. No. _____

Signature

June 13, 2017
Date

Todd Spalding
Typed or printed name

Vice President, Head of Intellectual Property
Title

Telephone Number

[X] Terminal disclaimer fee under 37 CFR 1.20(d) included.

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.

SUPPLEMENTAL SHEET FOR TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	Docket Number (Optional) AXJ-114BUSCN3

Prior patent Nos. applicable to this terminal disclaimer (referenced on first page):

9,494,601
9,409,980
9,556,263

Electronic Patent Application Fee Transmittal

Application Number:	15260888
Filing Date:	09-Sep-2016
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Attorney Docket Number:	AXJ-114BUSCN3

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

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

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
STATUTORY OR TERMINAL DISCLAIMER	1814	1	160	160
		Total in USD ($)		**160**

Electronic Acknowledgement Receipt

EFS ID:	29483167
Application Number:	15260888
International Application Number:	
Confirmation Number:	2558
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3
Receipt Date:	13-JUN-2017
Filing Date:	09-SEP-2016
Time Stamp:	17:44:51
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		AXJ_114BUSCN3_Response_with_Courtesy_Copies.pdf	1529515 dce35b66ec3c6770fde24956732afed602d950d1	yes	36

	Multipart Description/PDF files in .zip description				
	Document Description	**Start**	**End**		
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1		
	Claims	2	3		
	Applicant Arguments/Remarks Made in an Amendment	4	36		
Warnings:					
Information:					
2	Assignee showing of ownership per 37 CFR 3.73	AXJ_114BUSCN3_Assignee_Ownership.pdf	122490 3d5728d5117028f3f3568ffa8e9a7423ceef2032	no	2
Warnings:					
Information:					
3	Terminal Disclaimer Filed	AXJ_114_BUSCN3_TD_over_patents.pdf	90254 b5f80a5c43307896d868432efe5ad6f450a45456	no	2
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	30674 1e5fad1b5ec487268a349b04ea43a908bff8f33f	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1772933		

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
	15/260,888	BELL ET AL.	

Document Code - DISQ	Internal Document – DO NOT MAIL

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed : 13 June, 2017	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:

PAMELA YOUNG

Technology Center: PLRC

Telephone: _____

U.S. Patent and Trademark Office

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/260,888	Filing Date 09/09/2016	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

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

APPLICATION AS AMENDED – PART II

AMENDMENT	06/13/2017	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 10	Minus	** 20	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**0**

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

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** 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.

LIE
TONI HAKIM

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

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

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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Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

133485 7590 06/28/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER
ROGERS, JAMES L

ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 06/28/2017

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3	2558

TITLE OF INVENTION: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

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	09/28/2017

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)

133485 7590 06/28/2017
Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

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.
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3	2558

TITLE OF INVENTION: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

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	09/28/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
ROGERS, JAMES L	1644	424-133100

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

Samsung Bioepis Exhibit No. 1002
Page 1343

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3	2558

133485 7590 06/28/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

EXAMINER	
ROGERS, JAMES L	

ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 06/28/2017

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

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

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

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

PTOL-85 (Rev. 02/11)

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

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

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 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. 15/260,888	Applicant(s) BELL ET AL.	
	Examiner JAMES ROGERS	Art Unit 1644	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 *6/13/2017*.
   ☐ 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 *1-10*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

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

   * Certified copies not received: _____.

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

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

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

Attachment(s)

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

/JAMES ROGERS/ Examiner, Art Unit 1644	/DANIEL E. KOLKER/ Supervisory Patent Examiner, Art Unit 1644

DETAILED ACTION

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

2. Applicant's Amendment/Response, filed 6/13/2017, is acknowledged.

3. Applicant's IDSs, filed 6/13/2017, are acknowledged and have been considered.

REASONS FOR ALLOWANCE

4. The following is an Examiner's Statement of Reasons for Allowance:

The claims are drawn to a method of treating a patient suffering from paroxysmal nocturnal hemoglobinuria (PNH) by administering to the patient an antibody that binds C5, wherein the antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

Applicant's arguments in the Response of 6/13/2017 to the outstanding Non-final OA of 4/11/2017 which included rejections under 35 USC 102, 35 USC 103 and double patenting rejections as well as a Request for Additional Information Under 37 CFR 1.105 in conjunction with the Declaration Pursuant to 37 CFR 1.132 by Dr. Laural Boone, dated May 11, 2017 (previously submitted in a related case 15/148,839), is persuasive to overcome all of the rejections for the following reasons:

(a) None of the applied references in the rejections recite using an antibody which comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4 as currently recited and one of skill in the art would not have been easily guided to making antibodies with these recited sequences.

(b) Applicant's Response to the Request for Additional Information is considered sufficient and persuasive to conclude that each of the clinical trials conducted by applicant referenced in the Request did not constitute "public use" under 35 USC 102(b) of the claimed invention as they were not accessible to the public. As applicant notes in the response, factors which are used to determine if an invention is accessible to the public are set out by the Federal Circuit in *Dey, L.P. v. Sunovion Pharmaceuticals, Inc.*, (No. 2012-1428, Fed. Cir. May 20, 2013). The factual circumstances cited by applicant considered by the examiner as being most persuasive to negate any determination that the referenced clinical trials constituted "public use" is the use by applicant of confidentiality provisions and the fact that neither doctors nor patients had any knowledge of the currently claimed sequences of the antibody used in the studies. Other factual evidence presented by applicant includes the declaration by Laural S. Boone that strict control over the eculizumab drug was exercised for each clinical trial referenced (see p. 6 of the Declaration).

(c) The Double Patenting rejections have been overcome in light of applicant's approved TDs of 6/13/2017 over the '601, '980 and '263 patents.

Accordingly, claims 1-10, are deemed allowable.

5. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James Rogers whose telephone number is (571) 270-5374. The examiner can normally be reached Monday through Friday from 8:00 am to 5:30 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Daniel E. Kolker** can be reached on (571) 272-3181. The fax number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

June 21, 2017

/JAMES ROGERS/
Examiner, Art Unit 1644

/DANIEL E. KOLKER/

Supervisory Patent Examiner, Art Unit 1644

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

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

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

Application Number	15260888
Filing Date	2016-09-09
First Named Inventor	Leonard BELL
Art Unit	1644
Examiner Name	J. L. Rogers
Attorney Docket Number	AXJ-114BUSCN3

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20170015741	A1	2017-01-19	Leonard BELL	

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FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

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

EFS Web 2.1.17

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

	1		

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

EXAMINER SIGNATURE

Examiner Signature	/JAMES L ROGERS/	Date Considered	06/21/2017

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

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

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	15260888
	Filing Date	2016-09-09
	First Named Inventor	Leonard BELL
	Art Unit	1644
	Examiner Name	J. L. Rogers
	Attorney Docket Number	AXJ-114BUSCN3

CERTIFICATION STATEMENT

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

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

OR

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

See attached certification statement.

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

☒ A certification statement is not submitted herewith.

SIGNATURE

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

Signature	/Jill Gorny Sloper, Esq./	Date (YYYY-MM-DD)	2017-06-13
Name/Print	Jill Gorny Sloper, Esq.	Registration Number	60,760

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

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

Privacy Act Statement

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

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

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

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

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

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

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

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

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

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

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

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

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: June 13, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Leonard Bell *et al.*

Application No.: 15/260,888

Confirmation No.: 2558

Filed: September 9, 2016

Art Unit: 1644

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Examiner: J. L. Rogers

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (SIDS)

Dear Sir:

In compliance with 37 C.F.R. § 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the document listed on the attached PTO/SB/08. In accordance with 37 C.F.R. § 1.98(a)(2)(i)-(iv), Applicant has not included copies of U.S. patent publication.

It is respectfully requested that the document listed on the PTO/SB/08 be expressly considered by the Examiner during the prosecution of this application, and that the document be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

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

Applicant provides herewith copies of Office Actions from Applications previously made of record:

Office Actions (copies enclosed)				
Examiner's Initials	Serial No.:	Date Mailed from USPTO	Examiner	Docket No.
	15/284,015	04-11-2017	J. Rogers	AXJ-114BUSCN4
	15/284,015	11-01-2016	J. Rogers	AXJ-114BUSCN4
	15/148,839	06-07-2017	J. Rogers	AXJ-114BUSCN2
	15/148,839	04-04-2017	J. Rogers	AXJ-114BUSCN2

/JAMES L ROGERS/ 06/21/2017

The Examiner is requested to review the file histories of these applications, including cited references, Office Actions, Responses, etc., and is asked to contact Applicant's Attorney if the Examiner would like the Applicant to supply copies of any or all of the information included in these applications. If Applicant's Attorney is not contacted by the Examiner with such a request, then it will be concluded that the Examiner has reviewed or will review the file content of these applications.

Applicant respectfully requests that the Examiner initial the blank columns next to the cited Office Actions, to indicate that the information has been considered by the Examiner. Alternatively, Applicant requests that the Examiner insert the phrase, "All references considered except where lined through," on each page of the Information Disclosure Statement, along with the Examiner's initials.

The filing of this Information Disclosure Statement is not to be interpreted as a representation that the cited document is material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of the document herein be construed *per se* as a representation that such document is prior art. Moreover, Applicant understands the Examiner will make an independent evaluation of the cited document.

This Information Disclosure Statement is filed after the mailing date of a first Office Action on the merits, but before the mailing date of any of a Final Action under 37 C.F.R.§ 1.113, a Notice

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

Samsung Bioepis Exhibit No. 1002
Page 1354

of Allowance under 37 C.F.R. § 1.311 or an action that otherwise closes prosecution in this application (37 C.F.R. § 1.97(c)).

Please charge our Deposit Account No. 12-0080 in the amount of $180.00 covering the fee set forth in 37 C.F.R. § 1.17(p). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3.

Dated: June 13, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 742-4214 (Fax)
Attorney/Agent For Applicant

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

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15260888	BELL ET AL.
	Examiner	Art Unit
	JAMES ROGERS	1644

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
STN: DISSABS MEETINGS BIOSCI CAPLUS MEDICINE	10/19/2016	JR
EAST: PGPUB, USPAT, JPO, EPO, INVENTOR SEARCH	10/19/2016	JR
updated	6/21/2017	JR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
	USPAT, USPAD, SEQ ID NO: 2, 4	10/19/2016	JR

/JAMES ROGERS/ Examiner.Art Unit 1644	

EAST Search History

EAST Search History (Prior Art)

<This search history is empty>

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	14	(((hemolytic near3 disease) or hemolysis or hemoglobinuria) near20 (C5 near10 antibody)).clm.	US-PGPUB; USPAT	OR	ON	2017/06/21 12:20

6/21/2017 12:21:50 PM

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15260888	BELL ET AL.
	Examiner	**Art Unit**
	JAMES ROGERS	1644

CPC							
Symbol						**Type**	**Version**
C07K		16	/	18		F	2013-01-01
A61K		31	/	7105		I	2013-01-01
A61K		2039	/	505		A	2013-01-01
A61K		38	/	00		A	2013-01-01
C07K		2317	/	24		A	2013-01-01
C07K		2317	/	565		A	2013-01-01
A61K		9	/	0019		I	2013-01-01
C07K		2317	/	51		A	2013-01-01
C07K		2317	/	515		A	2013-01-01
			/				
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			/				
			/				
			/				

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

/JAMES ROGERS/ Examiner.Art Unit 1644		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	10	
/DANIEL E KOLKER/ Supervisory Patent Examiner.Art Unit 1644	06/21/2017	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15260888	BELL ET AL.
	Examiner	**Art Unit**
	JAMES ROGERS	1644

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION										
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED					
		A	6	1	K	39 / 00 (2006.01.01)						
CROSS REFERENCE(S)		A	6	1	K	9 / 00 (2006.0)						
		C	0	7	K	16 / 18 (2006.0)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)	C	0	7	K	19 / 00 (2006.0)						

/JAMES ROGERS/ Examiner.Art Unit 1644 (Assistant Examiner)	(Date)	**Total Claims Allowed:** 10	
/DANIEL E KOLKER/ Supervisory Patent Examiner.Art Unit 1644 (Primary Examiner)	06/21/2017 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15260888	BELL ET AL.
	Examiner	**Art Unit**
	JAMES ROGERS	1644

☒ 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

/JAMES ROGERS/ Examiner.Art Unit 1644 (Assistant Examiner)	(Date)	**Total Claims Allowed:** 10	
/DANIEL E KOLKER/ Supervisory Patent Examiner.Art Unit 1644 (Primary Examiner)	06/21/2017 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

PART B–FEE(S) TRANSMITTAL

Complete and send this form, together with the applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22314-1450
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)

133485 7590 06/28/2017
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127

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)
(S-signature)
(Date)

APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
15/260,888	09/09/2016	Leonard BELL	AXJ-114BUSCN3	2558

TITLE OF INVENTION: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

APPLN: TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960.00	$0	$0	$963.00	09/28/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
J. L. Rogers	1644	424-133100

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

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

[x] "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 attorney or agent. If no name is listed, no name will be printed

1. J. Darrell Fontenot, Ph.D., J.D.
2. Jane E. Remillard, Esq.
3. Jill Gorny Sloper, Esq.

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.

1a. NAME OF ASSIGNEE: Alexion Pharmaceuticals, Inc.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): New Haven, Connecticut

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

4a. The following fee(s) are submitted:

[x] Issue Fee

[ ] Publication Fee (No small entity discount permitted)

[x] Advance Order - # of Copies 1

4b. Payment of Fees(s): **(Please first reapply and previously paid fee shown above)**

[ ] A check is enclosed

[ ] Payment be credit card. Form PTO-2038 is attached

[x] The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 12-0080 (enclose an extra copy of this form)

5. Change of 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 regnlar undiscounted fee status. NOTE: Checking this box will be taken to be 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 /Jill Gorny Sloper, Esq./ Date June 28, 2017

Typed or printed name Jill Gorny Sloper, Esq. Registration No. 60,760

Samsung Bioepis Exhibit No. 1002
Page 1361

PTO/SB/47 (03-09)
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P.O. Box 1450
Alexandria, VA 22313-1450

- OR -

Fax to:
571-273-6500

INSTRUCTIONS: The issue fee must have been paid for application(s) listed on this form. In addition, only an address represented by a Customer Number can be established as the fee address for maintenance fee purposes (hereafter, fee address). A fee address should be established when correspondence related to maintenance fees should be mailed to a different address than the correspondence address for the application. **When to check the first box below:** If you have a Customer Number to represent the fee address. **When to check the second box below:** If you have no Customer Number representing the desired fee address, in which case a completed Request for Customer Number (PTO/SB/125) must be attached to this form. For more information on Customer Numbers, see the Manual of Patent Examining Procedure (MPEP) § 403.

For the following listed application(s), please recognize as the "Fee Address" under the provisions of 37 CFR 1.363 the address associated with:

[X] Customer Number: 127532

OR

[ ] The attached Request for Customer Number (PTO/SB/125) form.

PATENT NUMBER (if known)	APPLICATION NUMBER
	15/260,888

Completed by (check one):

[ ] Applicant/Inventor

[X] Attorney or Agent of record 60,760 (Reg. No.)

[ ] Assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

[ ] Assignee recorded at Reel _____ Frame _____

/Jill Gorny Sloper, Esq./
Signature

Jill Gorny Sloper, Esq.
Typed or printed name

(617) 217-4630
Requester's telephone number

June 28, 2017
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more that one signature is required, see below*.

[ ] * Total of 1 forms are submitted.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: June 28, 2017 Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Electronic Patent Application Fee Transmittal

Application Number:	15260888
Filing Date:	09-Sep-2016
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Attorney Docket Number:	AXJ-114BUSCN3

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	1501	1	960	960

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
Printed copy of patent - no color	8001	1	3	3
		Total in USD ($)		**963**

Electronic Acknowledgement Receipt	
EFS ID:	29631671
Application Number:	15260888
International Application Number:	
Confirmation Number:	2558
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3
Receipt Date:	28-JUN-2017
Filing Date:	09-SEP-2016
Time Stamp:	15:56:37
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$963
RAM confirmation Number	062917INTEFSW00002943120080
Deposit Account	120080
Authorized User	Jill Sloper
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 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	Issue Fee Payment (PTO-85B)	IFT.pdf	119819 8f70c5e40d18de7b524fc56bf94f3bc944a0cdbe	no	1
Warnings:					
Information:					
2	Change of Address	Change_Fee.pdf	122136 6108b391150fcc582d7289caad988d5a1613a882	no	1
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	32390 fae13ec15d3262d4ffa123aef0f6ebbde6baf21d	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	274345		

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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

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.

To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 133485

Jun 28, 2017 03:41:03 AM

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Application	Document	Mailroom Date	Attorney Docket No.
15260888	NOA	06/28/2017	AXJ-114BUSCN3
	1449	06/28/2017	AXJ-114BUSCN3
	1449	06/28/2017	AXJ-114BUSCN3

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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/260,888	08/08/2017	9725504	AXJ-114BUSCN3	2558

133485 7590 07/19/2017

Nelson Mullins Riley & Scarborough LLP/Alexion
One Post Office Square
30th Floor
Boston, MA 02109-2127

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

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

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

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

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

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

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

Leonard BELL, Woodbridge, CT;
Alexion Pharmaceuticals, Inc., New Haven, CT;
Russell P. ROTHER, Oklahoma City, OK;
Mark J. EVANS, Radnor, PA;

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To: chris.schlauch@nelsonmullins.com,ipqualityassuranceboston@nelsonmullins.com,ipboston.docketing@nel
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Application	Document	Mailroom Date	Attorney Docket No.
15260888	ISSUE.NTF	07/19/2017	AXJ-114BUSCN3

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9,725,504
APPLICATION NO.	:	15/260,888
ISSUE DATE	:	August 8, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

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

On page 2, first column, under “Other Publications”, line 25, delete “nocturnal; hemogloinuria” and insert - - nocturnal hemoglobinuria - -, therefor.

On page 3, first column, under “Other Publications”, line 38, delete “Neisseia” and insert - - Neisseria - -, therefor.

On page 3, second column, under “Other Publications”, line 33, delete “Hemoglobinuira” and insert - - Hemoglobinuria - -, therefor.

On page 3, second column, under “Other Publications”, line 37, delete “Phamaceuticals:” and insert - - Pharmaceuticals: - -, therefor.

On page 4, first column, under “Other Publications”, line 23, delete “Pharmaceuitcals” and insert - - Pharmaceuticals - -, therefor.

On page 4, first column, under “Other Publications”, line 53, delete “Endonuclial” and insert - - Endothelial - -, therefor.

On page 4, second column, under “Other Publications”, line 12, delete “EMAIHMPC” and insert - - EMA/HMPC - -, therefor.

On page 4, second column, under “Other Publications”, line 34, delete “htto:” and insert - - http: - -, therefor.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
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NELSON MULLINS RILEY & SCARBOROUGH LLP 1
One Post Office Square
Boston, Massachusetts 02109-2127

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APPLICATION NO.	:	15/260,888
ISSUE DATE	:	August 8, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

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

In column 2, line 65, delete “embodiment,” and insert - - embodiments, - -, therefor.

In column 4, line 2, delete “antibodies;” and insert - - antibodies, - -, therefor.

In column 4, line 23, delete “comprises,” and insert - - comprises - -, therefor.

In column 4, line 28, delete “the” and insert - - an - -, therefor.

In column 4, line 43, delete “No:5,” and insert - - No: 5, - -, therefor.

In column 4, line 43, delete “No:6,” and insert - - No: 6, - -, therefor.

In column 4, line 44, delete “No:7,” and insert - - No: 7, - -, therefor.

In column 4, line 47, delete “No:8,” and insert - - No: 8, - -, therefor.

In column 4, line 47, delete “No:9,” and insert - - No: 9, - -, therefor.

In column 4, line 47, delete “SEQ, ID NO:10.” and insert - - SEQ ID NO: 10. - -, therefor.

In column 4, line 65, delete “lass” and insert - - less - -, therefor.

In column 5, line 45, delete “life” and insert - - Life - -, therefor.

In column 6, line 24, delete “art,” and insert - - art. - -, therefor.

In column 6, line 25, delete “on” and insert - - or - -, therefor.

In column 6, line 35, delete “HSF S2” and insert - - HSP S2 - -, therefor.

In column 7, line 39, delete “pathway),” and insert - - pathway) - -, therefor.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 2
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Boston, Massachusetts 02109-2127

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9,725,504
APPLICATION NO.	:	15/260,888
ISSUE DATE	:	August 8, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

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

In column 7, line 61, delete “T,” and insert - - I, - -, therefor.

In column 7, line 65, delete “75 µg/ml,” and insert - - 75 µg/mL - -, therefor.

In column 7, line 67, delete “999 amino,” and insert - - 999 amino - -, therefor.

In column 8, line 26, delete “1975;” and insert - - 1978; - -, therefor.

In column 8, line 54, delete “al.;” and insert - - al., - -, therefor.

In column 9, line 14, delete “formation, of” and insert - - formation of - -, therefor.

In column 9, line 35, delete “as death” and insert - - as 1, death - -, therefor.

In column 10, line 31, delete “macro molecule” and insert - - macromolecule - -, therefor.

In column 10, line 42, delete “of” and insert - - or - -, therefor.

In column 11, line 42, delete “2d” and insert - - 2nd - -, therefor.

In column 12, line 7, delete “levels,” and insert - - levels - -, therefor.

In column 12, line 9, delete “BIA kit,” and insert - - EIA kit, - -, therefor.

In column 12, line 18, delete “anaphylatoxic” and insert - - anaphylatoxin - -, therefor.

In column 12, line 67, delete “fragments;” and insert - - fragments, - -, therefor.

In column 13, line 19, delete “tight” and insert - - light - -, therefor.

In column 13, line 19, delete “(CH1),” and insert - - (CH1) - -, therefor.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 3
One Post Office Square
Boston, Massachusetts 02109-2127

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CERTIFICATE OF CORRECTION

PATENT NO.	:	9,725,504
APPLICATION NO.	:	15/260,888
ISSUE DATE	:	August 8, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

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

In column 13, line 33, delete “scFV” and insert - - scFv - -, therefor.

In column 14, line 25, delete “(see, U.S.” and insert - - (see, e.g., U.S. - -, therefor.

In column 14, line 64, delete “from,” and insert - - from - -, therefor.

In column 14, line 66, delete “same/anti-human” and insert - - same anti-human - -, therefor.

In column 15, line 45, delete “&.” and insert - - & - -, therefor.

In column 16, line 47, delete “Philadelphia;” and insert - - Philadelphia, - -, therefor.

In column 17, line 62, delete “another,” and insert - - another - -, therefor.

In column 17, line 63, delete “liquid,” and insert - - liquid - -, therefor.

In column 19, line 2, after “(mg/mL)]” insert - - . - -.

In column 19, line 6, delete “antibodies,” and insert - - antibodies - -, therefor.

In column 19, line 25, delete “antibody,” and insert - - antibody - -, therefor.

In column 19, line 44, delete “syringes;” and insert - - syringes, - -, therefor.

In column 20, line 2, delete “genetically,” and insert - - genetically - -, therefor.

In column 20, lines 3-4, delete “Meningitides.” and insert - - meningitides. - -, therefor.

In column 20, line 24, delete “PBRC” and insert - - PRBC - -, therefor.

In column 20, line 31, delete “individuals” and insert - - individual's - -, therefor.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 4
One Post Office Square
Boston, Massachusetts 02109-2127

PTO/SB/44 (09-07)
Approved for use through 01/31/2020. OMB 0651-0033
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(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9,725,504
APPLICATION NO.	:	15/260,888
ISSUE DATE	:	August 8, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

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

In column 20, line 65, delete “QoL,” and insert - - QoL - -, therefor.

In column 21, line 3, delete “baseline,” and insert - - baseline - -, therefor.

In column 21, line 59, delete “cohorts;” and insert - - cohorts: - -, therefor.

In column 21, lines 62-63, delete “myelodysplasia” and insert - - myelodysplastic - -, therefor.

In column 22, line 11, delete “Pharmacodynamics.” and insert - - Pharmacodynamics - -, therefor.

In column 22, line 49, delete “above,” and insert - - above - -, therefor.

In column 22, line 56, delete “(standard” and insert - - (±standard - -, therefor.

In column 22, line 63, delete “type II” and insert - - type III - -, therefor.

In column 23, line 19, delete “(P=0.001),” and insert - - (P<0.001), - -, therefor.

In column 23, line 28, delete “patients,” and insert - - patients - -, therefor.

In columns 23-24, in Table 1, line 17, delete “2-sidexl” and insert - - 2-sided - -, therefor.

In column 24, line 6, delete “1.2 from” and insert - - 1.2 points from - -, therefor.

In column 24, line 17, delete “(P<0:001),” and insert - - (P<0.001), - -, therefor.

In column 24, line 21, delete “improvements,” and insert - - improvements - -, therefor.

In column 25, line 29, delete “analysis” and insert - - analysis, - -, therefor.

In column 25, line 30, delete “levels” and insert - - levels, - -, therefor.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 5
One Post Office Square
Boston, Massachusetts 02109-2127

PTO/SB/44 (09-07)
Approved for use through 01/31/2020. OMB 0651-0033
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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9,725,504
APPLICATION NO.	:	15/260,888
ISSUE DATE	:	August 8, 2017
INVENTOR(S)	:	Leonard BELL *et al.*

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

In column 25, in Table 3, line 49, delete “Categery” and insert - - Category - -, therefor.

In column 26, lines 1-2, delete “(treatment” and insert - - treatment - -, therefor.

In column 26, line 62, after “treated” insert - - patients - -.

In column 28, line 10, delete “patient,” and insert - - patient - -, therefor.

In column 28, line 54, delete “gene,” and insert - - gene - -, therefor.

In column 28, line 58, delete “PIG-A gene,” and insert - - PIG-A gene. - -, therefor.

In column 29, line 61, delete “Ref Type: Abstract”.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 6
One Post Office Square
Boston, Massachusetts 02109-2127

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 CFR § 1.6(a)(4).

Dated: September 22, 2017
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Leonard Bell *et al.*

Patent No.: 9,725,504

Issued: August 8, 2017

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Attention: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
PURSUANT TO 37 C.F.R. § 1.323

Dear Sir:

Upon reviewing the above-identified patent, Patentee noted typographical errors which should be corrected.

On page 2, first column, under "Other Publications", line 25, delete "nocturnal; hemogloinuria" and insert - - nocturnal hemoglobinuria - -, therefor.

On page 3, first column, under "Other Publications", line 38, delete "Neisseia" and insert - - Neisseria - -, therefor.

On page 3, second column, under "Other Publications", line 33, delete "Hemoglobinuira" and insert - - Hemoglobinuria - -, therefor.

On page 3, second column, under "Other Publications", line 37, delete "Phamaceuticals:" and insert - - Pharmaceuticals: - -, therefor.

On page 4, first column, under "Other Publications", line 23, delete "Pharmaceuitcals" and insert - - Pharmaceuticals - -, therefor.

On page 4, first column, under "Other Publications", line 53, delete "Endonuclial" and insert - - Endothelial - -, therefor.

On page 4, second column, under "Other Publications", line 12, delete "EMAIHMPC" and insert - - EMA/HMPC - -, therefor.

On page 4, second column, under "Other Publications", line 34, delete "htto:" and insert - - http: - -, therefor.

In the Specification:

In column 2, line 65, delete "embodiment," and insert - - embodiments, - -, therefor.

In column 4, line 2, delete "antibodies;" and insert - - antibodies, - -, therefor.

In column 4, line 23, delete "comprises," and insert - - comprises - -, therefor.

In column 4, line 28, delete "the" and insert - - an - -, therefor.

In column 4, line 43, delete "No:5," and insert - - No: 5, - -, therefor.

In column 4, line 43, delete "No:6," and insert - - No: 6, - -, therefor.

In column 4, line 44, delete "No:7," and insert - - No: 7, - -, therefor.

In column 4, line 47, delete "No:8," and insert - - No: 8, - -, therefor.

In column 4, line 47, delete "No:9," and insert - - No: 9, - -, therefor.

In column 4, line 47, delete "SEQ, ID NO:10." and insert - - SEQ ID NO: 10. - -, therefor.

In column 4, line 65, delete "lass" and insert - - less - -, therefor.

In column 5, line 45, delete "life" and insert - - Life - -, therefor.

In column 6, line 24, delete "art," and insert - - art. - -, therefor.

In column 6, line 25, delete "on" and insert - - or - -, therefor.

In column 6, line 35, delete "HSF S2" and insert - - HSP S2 - -, therefor.

In column 7, line 39, delete "pathway)," and insert - - pathway) - -, therefor.

In column 7, line 61, delete "T," and insert - - I, - -, therefor.

In column 7, line 65, delete "75 μg/ml," and insert - - 75 μg/mL - -, therefor.

In column 7, line 67, delete "999 amino," and insert - - 999 amino - -, therefor.

In column 8, line 26, delete "1975;" and insert - - 1978; - -, therefor.

In column 8, line 54, delete "al.;" and insert - - al., - -, therefor.

In column 9, line 14, delete "formation, of" and insert - - formation of - -, therefor.

In column 9, line 35, delete "as death" and insert - - as 1, death - -, therefor.

In column 10, line 31, delete "macro molecule" and insert - - macromolecule - -, therefor.

In column 10, line 42, delete "of" and insert - - or - -, therefor.

In column 11, line 42, delete "2d" and insert - - 2nd - -, therefor.

In column 12, line 7, delete "levels," and insert - - levels - -, therefor.

In column 12, line 9, delete "BIA kit," and insert - - EIA kit, - -, therefor.

In column 12, line 18, delete "anaphylatoxic" and insert - - anaphylatoxin - -, therefor.

In column 12, line 67, delete "fragments;" and insert - - fragments, - -, therefor.

In column 13, line 19, delete "tight" and insert - - light - -, therefor.

In column 13, line 19, delete "(CH1)," and insert - - (CH1) - -, therefor.

In column 13, line 33, delete "scFV" and insert - - scFv - -, therefor.

In column 14, line 25, delete "(see, U.S." and insert - - (see, e.g., U.S. - -, therefor.

In column 14, line 64, delete "from," and insert - - from - -, therefor.

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In column 15, line 45, delete "&." and insert - - & - -, therefor.

In column 16, line 47, delete "Philadelphia;" and insert - - Philadelphia, - -, therefor.

In column 17, line 62, delete "another," and insert - - another - -, therefor.

In column 17, line 63, delete “liquid,” and insert - - liquid - -, therefor.

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In column 19, line 6, delete “antibodies,” and insert - - antibodies - -, therefor.

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In column 20, lines 3-4, delete “Meningitides.” and insert - - meningitides. - -, therefor.

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In column 20, line 31, delete “individuals” and insert - - individual's - -, therefor.

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In column 22, line 56, delete “(standard” and insert - - (±standard - -, therefor.

In column 22, line 63, delete “type II” and insert - - type III - -, therefor.

In column 23, line 19, delete “(P=0.001),” and insert - - (P<0.001), - -, therefor.

In column 23, line 28, delete “patients,” and insert - - patients - -, therefor.

In columns 23-24, in Table 1, line 17, delete “2-sidexl” and insert - - 2-sided - -, therefor.

In column 24, line 6, delete “1.2 from” and insert - - 1.2 points from - -, therefor.

In column 24, line 17, delete “(P<0:001),” and insert - - (P<0.001), - -, therefor.

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In column 26, line 62, after "treated" insert - - patients - -.

In column 28, line 10, delete "patient," and insert - - patient - -, therefor.

In column 28, line 54, delete "gene," and insert - - gene - -, therefor.

In column 28, line 58, delete "PIG-A gene," and insert - - PIG-A gene. - -, therefor.

In column 29, line 61, delete "Ref Type: Abstract".

The errors now sought to be corrected are typographical errors the correction of which does not involve new matter or require reexamination.

Transmitted herewith is a proposed Certificate of Correction effecting such amendment. Patentee respectfully solicits the granting of the requested Certificate of Correction.

Please charge our Deposit Account No. 12-0080 in the amount of $100.00 covering the fee set forth in 37 C.F.R. § 1.20(a). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0080, under Order No. AXJ-114BUSCN3.

Dated: September 22, 2017

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Applicant

Electronic Patent Application Fee Transmittal

Application Number:	15260888
Filing Date:	09-Sep-2016
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Attorney Docket Number:	AXJ-114BUSCN3

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Certificate of correction	1811	1	100	100

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**100**

Electronic Acknowledgement Receipt	
EFS ID:	30439063
Application Number:	15260888
International Application Number:	
Confirmation Number:	2558
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Danielle Gamache
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3
Receipt Date:	22-SEP-2017
Filing Date:	09-SEP-2016
Time Stamp:	15:43:24
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$100
RAM confirmation Number	092517INTEFSW00003040120080
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Certificate of Correction	AXJ_114BUSCN3_Proposed_CoC.pdf	159699 86d6f17d18c99d8cdc9bd61b1db43cfc672d5424	no	6
Warnings:					
Information:					
2	Request for Certificate of Correction	AXJ_114BUSCN3_Request_CoC.pdf	215684 d48617e65c9d06b72b1855f0576432398c09d5eb	no	5
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30618 87ec0de752cf049bca51190b4a41dafcc099c964	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	406001		

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

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/260,888	Filing Date 09/09/2016	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

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

APPLICATION AS AMENDED – PART II

AMENDMENT	01/09/2017	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 10	Minus	** 20	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**0**

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

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** 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.

SLIE
HELENA PAYTON

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,725,504 B2
APPLICATION NO. : 15/260888
DATED : August 8, 2017
INVENTOR(S) : Leonard Bell et al.

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

On the Title Page

On page 2, first column, item (56) under "Other Publications", Line 25, delete "nocturnal; hemogloinuria" and insert -- nocturnal hemoglobinuria --, therefor.

On page 3, first column, item (56) under "Other Publications", Line 38, delete "Neisseia" and insert -- Neisseria --, therefor.

On page 3, second column, item (56) under "Other Publications", Line 33, delete "Hemoglobinuira" and insert -- Hemoglobinuria --, therefor.

On page 3, second column, item (56) under "Other Publications", Line 37, delete "Phamaceuticals:" and insert -- Pharmaceuticals: --, therefor.

On page 4, first column, item (56) under "Other Publications", Line 23, delete "Pharmaceuitcals" and insert -- Pharmaceuticals --, therefor.

On page 4, first column, item (56) under "Other Publications", Line 53, delete "Endonuclial" and insert -- Endothelial --, therefor.

On page 4, second column, item (56) under "Other Publications", Line 12, delete "EMAIHMPC" and insert -- EMA/HMPC --, therefor.

On page 4, second column, item (56) under "Other Publications", Line 34, delete "htto:" and insert -- http: --, therefor.

In the Specification

In Column 2, Line 65, delete "embodiment," and insert -- embodiments, --, therefor.

Signed and Sealed this
Thirty-first Day of October, 2017

Joseph Matal

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

In Column 4, Line 2, delete “antibodies;” and insert -- antibodies, --, therefor.

In Column 4, Line 23, delete “comprises,” and insert -- comprises --, therefor.

In Column 4, Line 28, delete “the” and insert -- an --, therefor.

In Column 4, Line 43, delete “No:5,” and insert -- No: 5, --, therefor.

In Column 4, Line 43, delete “No:6,” and insert -- No: 6, --, therefor.

In Column 4, Line 44, delete “No:7,” and insert -- No: 7, --, therefor.

In Column 4, Line 47, delete “No:8,” and insert -- No: 8, --, therefor.

In Column 4, Line 47, delete “No:9,” and insert -- No: 9, --, therefor.

In Column 4, Line 47, delete “SEQ, ID NO:10.” and insert -- SEQ ID NO: 10. --, therefor.

In Column 4, Line 65, delete “lass” and insert -- less --, therefor.

In Column 5, Line 45, delete “life” and insert -- Life --, therefor.

In Column 6, Line 24, delete “art,” and insert -- art. --, therefor.

In Column 6, Line 25, delete “on” and insert -- or --, therefor.

In Column 6, Line 35, delete “HSF S2” and insert -- HSP S2 --, therefor.

In Column 7, Line 39, delete “pathway),” and insert -- pathway) --, therefor.

In Column 7, Line 61, delete “T,” and insert -- I, --, therefor.

In Column 7, Line 65, delete “75 μg/ml,” and insert -- 75 μg/mL --, therefor.

In Column 7, Line 67, delete “999 amino,” and insert -- 999 amino --, therefor.

In Column 8, Line 26, delete “1975;” and insert -- 1978; --, therefor.

In Column 8, Line 54, delete “al.;” and insert -- al., --, therefor.

In Column 9, Line 14, delete “formation, of” and insert -- formation of --, therefor.

In Column 9, Line 35, delete “as death” and insert -- as 1, death --, therefor.

In Column 10, Line 31, delete “macro molecule” and insert -- macromolecule --, therefor.

In Column 10, Line 42, delete "of" and insert -- or --, therefor.

In Column 11, Line 42, delete "2d" and insert -- 2nd --, therefor.

In Column 12, Line 7, delete "levels," and insert -- levels --, therefor.

In Column 12, Line 9, delete "BIA kit," and insert -- EIA kit, --, therefor.

In Column 12, Line 18, delete "anaphylatoxic" and insert -- anaphylatoxin --, therefor.

In Column 12, Line 67, delete "fragments;" and insert -- fragments, --, therefor.

In Column 13, Line 19, delete "tight" and insert -- light --, therefor.

In Column 13, Line 19, delete "(CH1)," and insert -- (CH1) --, therefor.

In Column 13, Line 33, delete "scFV" and insert -- scFv --, therefor.

In Column 14, Line 25, delete "(see, U.S." and insert -- (see, e.g., U.S. --, therefor.

In Column 14, Line 64, delete "from," and insert -- from --, therefor.

In Column 14, Line 66, delete "same/anti-human" and insert -- same anti-human --, therefor.

In Column 15, Line 45, delete "&." and insert -- & --, therefor.

In Column 16, Line 47, delete "Philadelphia;" and insert -- Philadelphia, --, therefor.

In Column 17, Line 62, delete "another," and insert -- another --, therefor.

In Column 17, Line 63, delete "liquid," and insert -- liquid --, therefor.

In Column 19, Line 2, after "(mg/mL)]" insert -- . --.

In Column 19, Line 6, delete "antibodies," and insert -- antibodies --, therefor.

In Column 19, Line 25, delete "antibody," and insert -- antibody --, therefor.

In Column 19, Line 44, delete "syringes;" and insert -- syringes, --, therefor.

In Column 20, Line 2, delete "genetically," and insert -- genetically --, therefor.

In Column 20, Lines 3-4, delete "Meningitides." and insert -- meningitides. --, therefor.

In Column 20, Line 24, delete "PBRC" and insert -- PRBC --, therefor.

In Column 20, Line 31, delete "individuals" and insert -- individual's --, therefor.

In Column 20, Line 65, delete "QoL," and insert -- QoL --, therefor.

In Column 21, Line 3, delete "baseline," and insert -- baseline --, therefor.

In Column 21, Line 59, delete "cohorts;" and insert -- cohorts: --, therefor.

In Column 21, Lines 62-63, delete "myelodysplasia" and insert -- myelodysplastic --, therefor.

In Column 22, Line 11, delete "Pharmacodynamics." and insert -- Pharmacodynamics --, therefor.

In Column 22, Line 49, delete "above," and insert -- above --, therefor.

In Column 22, Line 56, delete "(standard" and insert -- (±standard --, therefor.

In Column 22, Line 63, delete "type II" and insert -- type III --, therefor.

In Column 23, Line 19, delete "(P=0.001)," and insert -- (P<0.001), --, therefor.

In Column 23, Line 28, delete "patients," and insert -- patients --, therefor.

In Columns 23-24, in Table 1, Line 17, delete "2-sidexl" and insert -- 2-sided --, therefor.

In Column 24, Line 6, delete "1.2 from" and insert -- 1.2 points from --, therefor.

In Column 24, Line 17, delete "(P<0:001)," and insert -- (P<0.001), --, therefor.

In Column 24, Line 21, delete "improvements," and insert -- improvements --, therefor.

In Column 25, Line 29, delete "analysis" and insert -- analysis, --, therefor.

In Column 25, Line 30, delete "levels" and insert -- levels, --, therefor.

In Column 25, in Table 3, Line 49, delete "Categery" and insert -- Category --, therefor.

In Column 26, Lines 1-2, delete "(treatment" and insert -- treatment --, therefor.

In Column 26, Line 62, after "treated" insert -- patients --.

In Column 28, Line 10, delete "patient," and insert -- patient --, therefor.

In Column 28, Line 54, delete "gene," and insert -- gene --, therefor.

In Column 28, Line 58, delete "PIG-A gene," and insert -- PIG-A gene. --, therefor.

In Column 29, Line 61, delete "Ref Type: Abstract".

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: May 15, 2018
Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

Docket No.: AXJ-114BUSCN3
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Letters Patent of:
Leonard Bell *et al.*

Patent No.: 9,725,504

Issued: August 8, 2017

For: TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT

Attention: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
PURSUANT TO 37 C.F.R. § 1.322

Dear Sir:

Upon reviewing the above-identified patent, Patentee noted a typographical error which should be corrected.

In the Sequence Listing:

At column 30, line number 31, delete

"SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSGK" and insert

- - SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK - -, therefor.

The error was not in the application as filed by applicant; accordingly, no fee is required.

Transmitted herewith is a proposed Certificate of Correction effecting such amendment. Patentee respectfully solicits the granting of the requested Certificate of Correction.

Dated: May 15, 2018

Respectfully submitted,

Electronic signature: /Jill Gorny Sloper, Esq./
Jill Gorny Sloper, Esq.
Registration No.: 60,760
NELSON MULLINS RILEY & SCARBOROUGH LLP
One Post Office Square
Boston, Massachusetts 02109-2127
(617) 217-4630
(617) 217-4699 (Fax)
Attorney/Agent For Patentee

PTO/SB/44 (09-07)
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(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 9,725,504

APPLICATION NO. : 15/260,888

ISSUE DATE : August 8, 2017

INVENTOR(S) : Leonard BELL *et al.*

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

At column 30, line number 31, delete

"SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSGK" and insert

- - SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK - -, therefor.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: May 15, 2018 Electronic Signature for Jill Gorny Sloper, Esq.: /Jill Gorny Sloper, Esq./

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Jill Gorny Sloper, Esq.
NELSON MULLINS RILEY & SCARBOROUGH LLP 1
One Post Office Square
Boston, Massachusetts 02109-2127

Electronic Acknowledgement Receipt

EFS ID:	32620104
Application Number:	15260888
International Application Number:	
Confirmation Number:	2558
Title of Invention:	TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT
First Named Inventor/Applicant Name:	Leonard BELL
Customer Number:	133485
Filer:	Jill R. Gorny Sloper/Carolyn Blake
Filer Authorized By:	Jill R. Gorny Sloper
Attorney Docket Number:	AXJ-114BUSCN3
Receipt Date:	15-MAY-2018
Filing Date:	09-SEP-2016
Time Stamp:	16:18:01
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Certificate of Correction	AXJ-114BUSCN3_Request-for-COC.pdf	26979 34eb55bdf3b42d60f5022dd06f8d06feb7e0f821	no	2

Warnings:

Information:					
2	Request for Certificate of Correction	AXJ-114BUSCN3_COC.pdf	24642 3a1025a0965069ded81ef2b1eab2ed03f535c2be	no	1
Warnings:					
Information:					
			Total Files Size (in bytes):	51621	

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

New Applications Under 35 U.S.C. 111

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

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

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

New International Application Filed with the USPTO as a Receiving Office

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

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO.	: 9,725,504 B2
APPLICATION NO.	: 15/260888
DATED	: August 8, 2017
INVENTOR(S)	: Leonard Bell et al.

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

In the Specification

At Column 30, Line number 31, delete
"SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSGK" and insert
-- SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK --, therefor.

Signed and Sealed this
Twelfth Day of June, 2018

Andrei Iancu
Director of the United States Patent and Trademark Office

Patent Trial and Appeal Board

A petition has been filed in Patent Number 9,725,504, Application Number 15/260888 on February 28, 2019.

The AIA Review Number is IPR2019-00739.

To view the documents filed in this petition, go to https://ptab.uspto.gov and Search for the AIA Review Number.

- Enter your search criteria on the "Search PTAB" page
- Type in the AIA Review Number or Patent Number
- You will need to answer the CAPTCHA to prove that you are not a robot.
- Click on the "Search" button
- The search results will appear identifying the AIA Review Number
- Click on the "View Documents" button
- A pop up window will appear with a list of documents
- Click on the "Download" button to download the document.

Questions regarding this notice should be directed to the Patent Trial and Appeal Board at 571-272-7822.

Trials@uspto.gov
571-272-7822

Paper 15
Entered: August 30, 2019

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

AMGEN INC.,
Petitioner,

v.

ALEXION PHARMACEUTICALS, INC.,
Patent Owner.

IPR2019-00739
Patent 9,725,504 B2

Before TINA E. HULSE, ROBERT A. POLLOCK, and RYAN H. FLAX, *Administrative Patent Judges*.

POLLOCK, *Administrative Patent Judge*.

DECISION
Institution of *Inter Partes* Review
35 U.S.C. § 314(a)

I. INTRODUCTION

Amgen Inc. ("Petitioner") filed a Petition for an *inter partes* review of claims 1–10 of U.S. Patent No. 9,725,504 B2 ("the '504 patent," Ex. 1001). Paper 2 ("Pet."). Alexion Pharmaceuticals ("Patent Owner" or "Alexion") timely filed a Preliminary Response. Paper 10 ("Prelim. Resp."). The parties further submitted an authorized Reply and Sur-Reply to the Preliminary Response. Paper 13 ("Reply"); Paper 14 ("Sur-Reply").

We review the Petition, Preliminary Response, Reply, Sur-Reply, and accompanying evidence under 35 U.S.C. § 314. An *inter partes* review may not be instituted unless "the information presented in the petition . . . and any response . . . shows that there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition." 35 U.S.C. § 314(a). Further, a decision to institute may not institute on fewer than all claims challenged in the petition. *SAS Inst., Inc. v. Iancu*, 138 S. Ct. 1348, 1359–60 (2018).

After considering the evidence and arguments presented in the Petition and Preliminary Response, Reply, and Sur-Reply, we determine that Petitioner demonstrates a reasonable likelihood of prevailing in showing that at least one of the challenged claims of the '504 patent is unpatentable. Accordingly, we institute an *inter partes* review as to all the challenged claims of the '504 patent on all the grounds of unpatentability set forth in the Petition.

A. Real Parties-in-Interest

Petitioner identifies only itself as the real party-in-interest. Pet. 73. Patent Owner, likewise, identifies only itself as the real party-in-interest. Paper 3, 2.

B. Related Proceedings

The '504 patent shares essentially the same specification with U.S. Patent Nos. 9,718,880 B2 ("the '880 patent) and 9,732,149 ("the '149 patent"). Amgen has filed Petitions for *Inter Partes* Review of the '504, '880, and '149 patents in IPR2019-00739, IPR2019-00740, and IPR2019-00741, respectively. Pet. 73–74; Paper 3, 2.

The '504, '880, and '149 patents are related as follows: The '149 patent issued from application No. 15,284,015, filed January 19, 2017, which is a continuation of application No. 15/260,888 (now the '504 patent), filed on September 9, 2016, which is a continuation of application No. 15/148,839 (now the '888 patent), filed on May 6, 2016, which is a continuation of application No. 13/426,973, filed on March 22, 2012, which is a continuation of application No. 12/225,040, filed as application No. PCT/US2007/006606 on March 15, 2007. The parties do not dispute that March 15, 2007, is the relevant priority date of the challenged patent.

C. The '504 patent and Relevant Background

The '504 patent relates to the use of a humanized anti-C5 antibody (eculizumab) for the treatment of paroxysmal nocturnal hemoglobinuria. *See* Ex. 1001, Abstract. For reference, we reproduce Figure 1 from the

Balthasar Declaration,[1] illustrating the basic structure of an antibody, such as eculizumab:

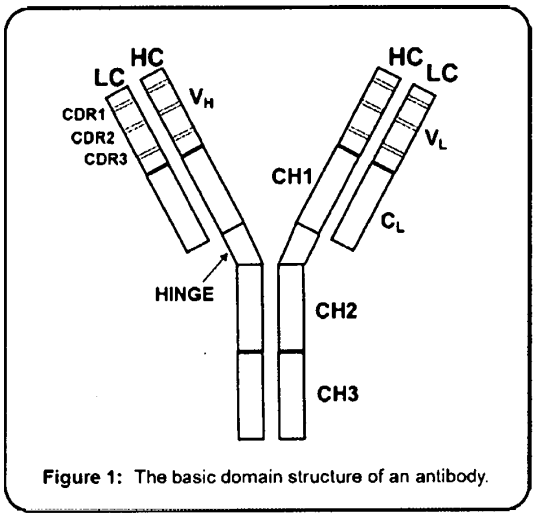


Figure 1: The basic domain structure of an antibody.

The figure above shows a basic antibody structure having hinged heavy chains (HC) and accompanying light chains (LC), each having constant regions (C_H and C_L) and variable regions (V_H and V_L), all arranged in a general "Y" shaped structure, as the variable regions and portions of the constant heavy chain regions are hinged away from one another. Ex. 1002 ¶¶ 23–24. The variable regions of each chain also include three complementarity determining regions (CDR), which provide the antibody with antigen-binding specificity. *Id.*

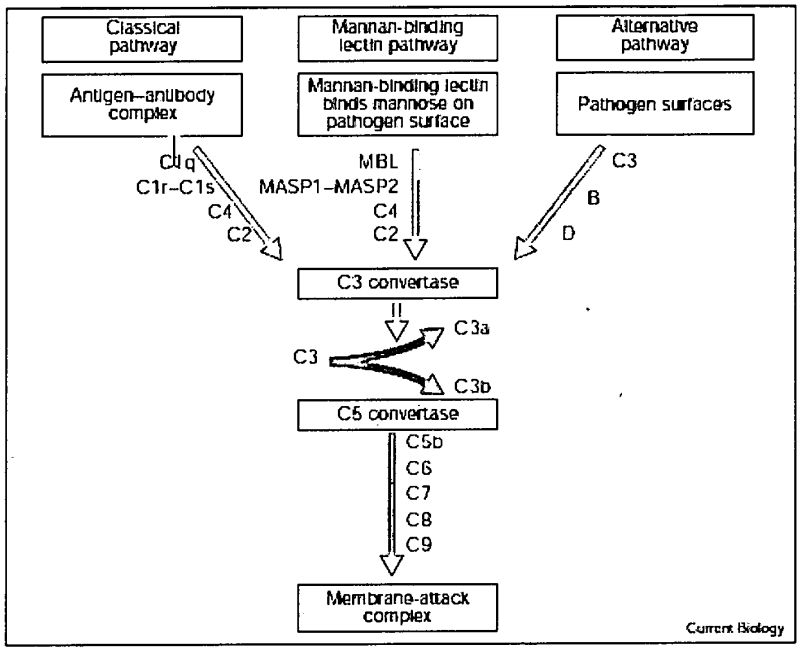
[1] Declaration of Dr. Joseph P. Balthasar, Ph.D. (Ex. 1002, "Balthasar Declaration").

Paroxysmal nocturnal hemoglobinuria or PNH is an acquired hemolytic disease resulting from loss of function in certain cytoprotective proteins. Ex. 1001, 1:27–35; Ex. 1002 ¶ 31. This loss of function renders red blood cells, platelets, and other blood cells highly sensitive to attack via activated complement proteins (explained in detail below). Ex. 1002 ¶ 31. The resultant complement-mediated lysis of blood cells results in anemia, hemoglobinuria, and related symptoms, which impair a patient's quality of life to the extent that "[m]any PNH patients depend on blood transfusions to maintain adequate erythrocyte hemoglobin levels." Ex. 1001, 1:42–53. As further explained by Petitioner's expert, Dr. Balthasar, "[a]s a result of the destruction of RBCs and the resultant release of free hemoglobin into the blood, 'PNH is characterized by hemolytic anemia (a decreased number of red blood cells), hemoglobinuria (the presence of hemoglobin in the urine particularly evident after sleeping), and hemoglobinemia (the presence of hemoglobin in the bloodstream.')" Ex. 1002 ¶ 32 (quoting Ex. 1005 ¶ 7).

"Complement" is a "system of plasma proteins . . . so-named because it complements the activity of antibody in the lysis of bacteria." Ex. 1022 R259; *see generally* Ex. 1001, 7:6–8:56. As part of the immune system, complement "has a central role in host defense against many micro-organisms and in the modulation of inflammatory reactions.'" *Id.*; *see*

Ex. 1002 ¶ 28. The figure below shows "the main pathways and components of the complement activation system." Ex. 1022, R259.

Figure 1



The above figure illustrates how various complement proteins are organized into the "classical," "mannan-binding," and "alternative" activation pathways. Ex. 1022, R259; *see* Ex. 1001, 7:17–19. All three pathways lead to the cleavage of C3 convertase and the resultant cleavage of C5 convertase into C5a and C5b. Ex. 1022, Fig. 1; *see* Ex. 1002 ¶ 29. As summarized in paragraph 29 of the Balthasar Declaration (Ex. 1002), cleavage of C5 initiates the terminal complement cascade. Conversely, blocking the cleavage of C5 prevents complement activation. *See, e.g.*, Ex. 1001, 10:65–

11:6 ("U.S. Pat. No. 6,353,245 [Evans][2] teaches an antibody which binds to C5 and inhibits cleavage into C5a and C5b thereby decreasing the formation not only of C5a but also the downstream complement components."); 12:21–29.

According to the Specification, eculizumab is a humanized monoclonal antibody directed against the terminal complement protein C5 convertase and, is thus, intended to suppress the terminal activation cascade and resultant complement activation. Ex. 1001, Abstract, 1:56–57 (citing Thomas C. Thomas et al., *Inhibition of Complement Activity by Humanized Anti-C5 Antibody and Single-Chain Fv*, 33(17) MOL. IMMUNOL. 1389–401 (1996) (Ex. 1023, "Thomas")). More specifically, "eculizumab" refers to humanized antibodies derived from mouse antibody 5G1.1, sometimes referred to as "murine 5G1.1" or "m5G1.1". *See* Prelim. Resp. 12–13. The term "humanized" refers to an antibody having a human framework, into which CDR regions from a non-human monoclonal antibody (e.g., mouse) are inserted. Ex. 1007, 5:57–67; Ex. 1002 ¶¶ 22–26. Accordingly, humanized versions of non-human antibodies may be indicated by the prefix "h" or "hu" as in "h5G1.1" and "hu5G1.1." *See e.g.*, Pet. 17, n.11, 19; Prelim. Resp. 12; Ex. 1002 ¶ 48.

Claim 1 refers to SEQ ID NO: 2 and SEQ ID NO: 4, which together comprise an eculizumab antibody. *See generally* Prelim. Resp. 1–2. Thus, for example, the '504 patent identifies SEQ ID NO: 2 and SEQ ID NO: 4 as the "Eculizumab Heavy Chain" and "Eculizumab Light Chain,"

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[2] US 6,355,245 B1, issued Mar. 12, 2002 (Ex. 1007).

respectively. Ex. 1001, 30:22–31, 38–44. It is undisputed that SEQ ID NO: 2 encodes a hybrid IgG2/IgG4 heavy chain (i.e., having a genetically engineered heavy chain constant region derived from portions of IgG2 and IgG4 isotype antibodies). *See, e.g.*, Pet. 3; Prelim. Resp. 14; Ex. 1033, 1258 (Figure 2). Eculizumab is the non-proprietary name for Patent Owner's SOLIRIS product, which was approved by the FDA "for treatment of patients with PNH." *See, e.g.,* Prelim. Resp. 1–2, 5–6 (citing Ex. 1033, 1256;[3] Ex. 2005, 1[4]); Pet. 2 (citing Ex. 1009, 2); *see also* Prelim. Resp. 16 ("The '504 patent claims recite the complete amino acid sequence for SOLIRIS® . . . the heavy chain consisting of SEQ ID NO: 2, and the light chain consisting of SEQ ID NO: 4. (AMG1001 at cols. 31–33, 35.).").

The '504 patent also discusses the conduct and results of the TRIUMPH trial in which 88 red blood cell transfusion dependent PNH patients were randomly assigned "to receive either placebo or Eculizumab (Soliris™, Alexion Pharmaceuticals, Inc.)." Ex. 1001, 19:51–28:38.

> Study medication was dosed in a blinded fashion as follows: 600 mg eculizumab for patients randomly assigned to active drug, or placebo for those patients randomly assigned to placebo, respectively via IV infusion every 7±1 days for 4 doses; followed by 900 mg eculizumab, or placebo, respectively, via IV infusion 7±1 day later; followed by a maintenance dose of 900 mg eculizumab, or placebo, respectively, via IV infusion every 14±2 days for a total of 26 weeks of treatment.

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[3] Rother et al., *"Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria,"* 25(11) NATURE BIOTECHNOLOGY 1256–1264 (2007).
[4] SOLIRIS Product Label (rev. 3/2007).

Id. at 20:43–50. The Specification concludes that "[t]he results of the TRIUMPH study indicate that terminal complement inhibition with eculizumab safely and effectively addresses an important consequence of the underlying genetic defect in PNH hematopoietic stem cells by providing a therapeutic replacement for the terminal complement inhibitor deficiency." *Id.* at 28:33–38. "[E]culizumab stabilized hemoglobin levels, decreased the need for transfusions, and improved quality of life in PNH patients via reduced intravascular hemolysis." *Id.* at Abstract.

D. Challenged Claims

Petitioner challenges claims 1–10 of the '504 patent, of which claim 1 is independent; claim 1 reads as follows:

> 1. A method of treating a patient suffering from paroxysmal nocturnal hemoglobinuria (PNH) comprising administering to the patient a pharmaceutical composition comprising an antibody that binds C5, wherein the antibody comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.

Ex. 1001, 39:2–7. Among the dependent claims, claim 2 requires administration of the compound by intravenous infusion, claims 3 and 8 relate to dosage and dosing protocol, claims 4–6 relate to single unit dosage forms, claim 7 requires that the patient is anemic, and claim 9 and its dependent claim, claim 10, require that "administration of the antibody results in an immediate and sustained decrease in mean levels of lactate dehydrogenase (LDH)."

E. Asserted Grounds of Unpatentability

Petitioner asserts the following seven grounds for unpatentability (Pet. 23):

Ground	Claim(s)	Basis	Asserted Reference(s)
1	1–3, 7–10	102(b)[5]	Hillmen[6]
2	4, 5	103(a)	Hillmen and Bell[7]
3	6	103(a)	Hillmen, Bell, and Wang[8]
4	1–5, 7–10	103(a)	Bell, Bowdish,[9] and Evans[10]
5	6	103(a)	Bell, Bowdish, Evans, and Wang
6	1–5, 7–10	103(a)	Bell, Evans, and Mueller[11]
7	6	103(a)	Bell, Evans, Mueller, and Wang

[5] The Leahy-Smith America Invents Act, Pub. L. No. 112-29, 125 Stat. 284 (2011) ("AIA"), amended 35 U.S.C. §§ 102 and 103. Because the challenged claims of the '504 patent have an effective filing date before the effective date of the applicable AIA amendments, we refer to the pre-AIA versions of 35 U.S.C. §§ 102 and 103 throughout this Decision.
[6] Hillmen et al., "*Effect of Eculizumab on Hemolysis and Transfusion Requirements in Patients with Paroxysmal Nocturnal Hemoglobinuria,*" 350(6) N. Engl. J. Med. 552–59 (2004). (Ex. 1004).
[7] Bell et al., US 2005/0191298 A1, published Sept. 1, 2005 (Ex. 1005).
[8] Wang, US 2005/0271660 Al, published Dec. 8, 2005 (Ex. 1028)
[9] Bowdish et al., US 2003/0232972 A1, published Dec. 18, 2003 (Ex. 1006).
[10] Evans et al., US 6,355,245 B1, issued March 12, 2002 (Ex. 1007).
[11] Mueller et al., WO 97/11971, published April 3, 1997 (Ex. 1008).

In support of its patentability challenges, Petitioner relies on, *inter alia*, the Declaration of Joseph P. Balthasar, Ph.D. Ex. 1002.

F. Overview of Asserted References

1. Overview of Hillmen (Ex. 1004)

Hillmen discloses that "[p]aroxysmal nocturnal hemoglobinuria (PNH) arises from a somatic mutation of the PIG-A gene in a hematopoietic stem cell and the subsequent production of blood cells with a deficiency of surface proteins that protect cells from attack by the complement system." Ex. 1004, 552.

> Patients with PNH have chronic, often disabling symptoms of fatigue and intermittent episodes of dysphagia, abdominal pain, and hemoglobinuria. These symptoms are thought to be related to the intravascular destruction of PNH type III erythrocytes, which are deficient in complement inhibitors, by autologous complement. The hemolytic anemia frequently renders the patients transfusion-dependent. In addition, patients have an extremely high risk of potentially life-threatening thrombosis, particularly thrombosis of the hepatic and cerebral veins. Approximately 50 percent of patients with PNH die of the disease; the median duration of survival after diagnosis is 10 years.

Id. at 557.

Hillmen reports on the efficacy of eculizumab for "reduc[ing] the incidence of intravascular hemolysis, hemoglobinuria, and transfusion requirements in patients with PNH." *Id.* at 553. "Eleven transfusion-dependent patients with PNH received infusions of eculizumab (600mg) every week for four weeks, followed one week later by a 900-mg dose and then by 900 mg every other week through week 12" *Id.* at Abstract, *see id.*

at 554. Based on this treatment, Hillmen concludes:

> Eculizumab is safe and well tolerated in patients with PNH. This antibody against terminal complement protein C5 reduces intravascular hemolysis, hemoglobinuria, and the need for transfusion, with an associated improvement in the quality of life in patients with PNH.

Id. at Abstract.

Hillmen characterizes eculizumab as "a recombinant humanized monoclonal antibody that was designed to block the activation of terminal complement components." *Id.* (citing, e.g., Thomas (Ex. 1023)). According to Hillmen, the antibody "binds specifically to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b, thereby preventing the release of the inflammatory mediator C5a and the formation of the cytolyticpore C5b-C9." *Id.* Because eculizumab "specifically prevents cleavage of C5, which is necessary for assembly of the membrane-attack complex," the treatment "presumably prolongs the survival of type III erythrocytes . . . which are highly sensitive to lysis by complement." *Id.* at 557.

2. Overview of Bell (Ex. 1005)

Bell discloses the treatment of PNH "using a compound which binds to or otherwise blocks the generation and/or activity of one or more complement components. . . . In particularly useful embodiments, the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb (eculizumab), h5G1.1-scFv (pexelizumab) and other functional fragments of hSGl.l." *Id.* ¶ 12; *see also id.* ¶ 52 ("The antibody h5G1.1-mAb is currently undergoing clinical trials under the tradename

eculizumab."). Bell further discloses: "Methods for the preparation of h5G1.1-mAb, h5G1.1-scFv and other functional fragments of h5G1.1 are described in [Evans] and [Thomas] . . . the disclosures of which are incorporated herein in their entirety." *Id.* ¶ 52.

As noted by Dr. Balthasar, the data disclosed in Bell overlaps with that in Hillmen, but further includes data on extension studies in which patients continued treatment for paroxysmal nocturnal hemoglobinuria for a total of two additional years. *See* Ex. 1002 ¶ 39 (citations omitted).[12] Briefly, eleven transfusion-dependent PNH patients received weekly 600 mg doses of eculizumab by infusion for four weeks, followed by "900 mg of eculizumab 1 week later[,] then 900 mg on a bi-weekly basis." Ex. 1005 ¶¶ 81–82. Bell characterizes the first twelve weeks of treatment as a "pilot study." *Id.* ¶ 82. "Following completion of the initial acute phase twelve week study, all patients participated in an extension study conducted to a total of 64 weeks. Ten of the eleven patients participated in an extension study conducted to a total of two years." *Id.* Bell concludes that "[p]atients in the two year study experienced a reduction in adverse symptoms associated with PNH. *Id.* ¶¶ 82, 96.

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[12] Page 38, footnote 17, of the Petition states: "Because the clinical study taught in Bell is the same C02-001 study disclosed in Hillmen, which discloses the eculizumab amino acid sequences of SEQ ID NOs: 2 and 4, Bell, too, therefore anticipates claims 1–4 and 7–10 for the same reasons discussed above for Hillmen." Despite this assertion, Petitioner's sole anticipation-based Ground in this IPR relies on Hillmen. *See* Pet. 23.

3. Overview of Wang (Ex. 1028)

Wang discloses formulations of eculizumab suitable for nebulization and pulmonary delivery. *See, e.g.*, ¶¶ 25, 62, 67. Wang discloses formulations comprising from 1 to 30 mg/ml eculizumab, and provides evidence that a formulation having 30 mg/ml eculizumab can be effectively and efficiently delivered using a conventional nebulizer. *Id.* ¶¶ 171–173, Fig. 10.

4. Overview of Bowdish (Ex. 1006)

Bowdish discloses "[i]mmunoglobulins or fragments thereof hav[ing] a peptide of interest inserted into a complementarity determining region (CDR) of an antibody molecule," whereupon, "[t]he antibody molecule serves as a scaffold for presentation of the peptide and confers upon the peptide enhanced stability." Ex. 1006 ¶ 6. In certain "embodiments, the peptide replacing the amino acids of a CDR is an agonist TPO [thrombopoeitin] peptide." *Id.* ¶ 17.

In Example 4, Bowdish describes a TPO mimetic peptide graft into the heavy chain CDR3 of antibody framework 5G1.1, described in Evans, which Bowdish incorporates by reference. *Id.* ¶¶ 191–193. According to Bowdish:

> Construction of 5G1.1 is described in U.S. Application. Ser. No. 08/487,283, incorporated herein by reference.[13] The sequence was cloned into 5G1.1 in such a fashion as to replace the native CDR3 . . . [wherein t]he peptide graft translated into amino acids is Leu Pro Ile Glu Gly Pro Thr Leu Arg Gln Trp Leu Ala Arg Ala

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[13] U.S. Application Ser. No. 08/487,283 matured into U.S. Patent No. 6,355,245 B1, referenced herein as Evans (Ex. 1007).

> Pro Val (SEQ. ID. NO: 66). The 5G1+peptide was produced as a whole IgG antibody (See FIGS. 13A and 13B).

Id. ¶ 191. "Purified 5G1.1+peptide antibody as well as the parental 5G1.1 were analyzed for their ability to bind to cMpl receptor by FACS analysis." *Id.* ¶ 192.

In SEQ ID NOs: 69 and 70, respectively, Bowdish discloses the amino acid and nucleotide sequences for the "5G1.1 Light Chain." In SEQ ID NO: 67, Bowdish discloses the amino acid sequence of the "5G1.1–TPO Heavy Chain," with the substituted TPO mimetic sequence marked in bold. An excerpt of that sequence showing the amino acids of the TPO substitution in bold reads: DTAVYYCAR**LPIEGPTLRQWLAARAPV** WGQGTLVTVSS. Bowdish discloses the corresponding nucleotide sequence in SEQ ID NO: 68.

5. Overview of Evans (Ex. 1007)

Evans discloses anti-C5 antibodies useful in the treatment of glomerulonephritis (GN). Ex. 1007, Abstract. Evans' Example 7 describes the isolation anti-C5 monoclonal antibodies from mouse hybridoma designated 5G1.1. *Id.* at 37:34–39:30. In Figures 18 and 19, respectively, Evans discloses the amino acid sequence of the light and heavy chain variable regions of mouse antibody 5G1.1, with "[t]he complementarity determining region (CDR) residues according to the sequence variability definition or according to the structural variability definition . . . [bolded] and [underlined], respectively." *Id.* at 9:65–10:20. A representation of an excerpt of the heavy chain sequence showing the amino acids of CDR3 so marked reads: DSAVYYCAR**YFFGSSPNWYFD**VWGAGTTVTVSS.

See id. at Fig. 19.

Evans describes making a series of different humanized 5G1.1 scFv[14] and full-length antibodies containing the CDR regions from the murine 5G1.1 antibody. AMG1007, 37:35-39:30, 42:59-45:33. With respect to the former, Evans discloses that "[p]articularly preferred constant regions . . . are IgG constant regions, which may be unaltered, or constructed of a mixture of constant domains from IgGs of various subtypes, e.g., IgG1 and IgG4." *Id.* at 45:29–33.

In Example 11, Evans discloses steps in the humanization of mouse antibody 5G1.1, including the construction of recombinant antibody, 5G1.1 scFv CO12. *Id.* at 42:59–45:33. According to Dr. Balthasar, the construct "'5G1.1 scFv CO12" contains all six 5G1.1 CDR regions and its sequence aligns perfectly with the eculizumab variable regions claimed by the '504 patent." Ex. 1002 ¶ 55 (comparing portions of SEQ ID NO: 2 of the '504 patent with portions of Evans' SEQ ID NO. 2). Dr. Balthasar's Figure 6, for example, shows that both sequences contain the larger of the two overlapping amino acid sequences Evans identifies as CDR3, YFFGSSPNWYFDV. *Id.*

6. Overview of Mueller (Ex. 1008)

Mueller discloses "[a]ntibodies to porcine P-selecting protein, porcine VCAM protein and porcine CD86 protein are useful for diagnosing human

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[14] As Dr. Balthasar notes, "a scFv comprises an antibody's light and heavy chain variable domains connected by a linker." Ex. 1002 ¶ 54, n.4 (citing Ex. 1007, 6:39-41).

rejection of porcine xenotransplants and for improving xenotransplantation of porcine, cells, tissues and organs into human recipients." Ex. 1008, Abstract. According to Mueller, one object of the invention is to provide antibody molecules that neither activate complement nor bind to the FC receptor. *Id.* at 7:28–31. To achieve these and other goals, Mueller points to "[r]ecombinant (chimeric and/or humanized) antibody molecules comprising the C1 and hinge regions of human IgG2 and the C2 and C3 regions of human IgG4, such antibodies being referred to hereinafter as 'HuG2/G4 mAb.'" *Id.* at 8:23–26. Mueller developed and tested "chimeric antibodies containing the C1 and hinge region of human IgG2 and the C2 and C3 regions of human IgG4." *Id.* at 12:19–33. As controls for these experiments, Mueller used "a humanized antibody directed against human C5 (h5G1.1 C012 HuG4 mAb)." *Id.* at 11:34–12:4, 12:34–13:2, Figures 11, 12, 15. On pages 58–61, Mueller discloses the cDNA and amino acid sequence of "Human G2/G4."

II. ANALYSIS

A. Principles of Law

"In an [*inter partes* review], the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable." *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3) (requiring *inter partes* review petitions to identify "with particularity . . . the evidence that supports the grounds for the challenge to each claim")). This burden of persuasion never shifts to Patent Owner. *See Dynamic Drinkware, LLC v. Nat'l Graphics,*

Inc., 800 F.3d 1375, 1378 (Fed. Cir. 2015) (discussing the burden of proof in *inter partes* review).

To establish anticipation, each and every element in a claim, arranged as recited in the claim, must be found in a single prior art reference. *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1369 (Fed. Cir. 2008); *Karsten Mfg. Corp. v. Cleveland Golf Co.*, 242 F.3d 1376, 1383 (Fed. Cir. 2001). This "single prior art reference must expressly or inherently disclose each claim limitation." *Finisar Corp. v. DirecTV Group, Inc.*, 523 F.3d 1323, 1334 (Fed. Cir. 2008). "Under the principles of inherency, if the prior art necessarily . . . includes[ ] the claimed limitations, it anticipates." *MEHL/Biophile Int'l Corp. v. Milgraum*, 192 F.3d 1362, 1365 (Fed. Cir. 1999). Similarly, "[a] reference anticipates a claim if it discloses the claimed invention 'such that a skilled artisan could take its teachings in combination with his own knowledge of the particular art and be in possession of the invention.'" *In re Graves*, 69 F.3d 1147, 1152 (Fed. Cir. 1995) (internal citation and emphasis omitted). Moreover, "it is proper to take into account not only specific teachings of the reference but also the inferences which one skilled in the art would reasonably be expected to draw therefrom." *In re Preda*, 401 F.2d 825, 826 (CCPA 1968); *see Eli Lilly v. Los Angeles Biomedical Res. Inst.*, 849 F.3d 1073, 1074–75 (Fed. Cir. 2017).

A claim is unpatentable under 35 U.S.C. § 103(a) 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 that

subject matter pertains. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) any differences between the claimed subject matter and the prior art; (3) the level of ordinary skill in the art; and (4) objective evidence of non-obviousness. *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

In analyzing the obviousness of a combination of prior art elements, it can be important to identify a reason that would have prompted one of skill in the art "to combine . . . known elements in the fashion claimed by the patent at issue." *KSR*, 550 U.S. at 418. A precise teaching directed to the specific subject matter of a challenged claim is not necessary to establish obviousness. *Id.* Rather, "any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed." *Id.* at 420. Accordingly, a party that petitions the Board for a determination of unpatentability based on obviousness must show that "a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so." *In re Magnum Oil Tools Int'l, Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (internal quotations and citations omitted).

B. Person of Ordinary Skill in the Art

In determining the level of skill in the art, we consider the type of problems encountered in the art, the prior art solutions to those problems, the rapidity with which innovations are made, the sophistication of the

technology, and the educational level of active workers in the field. *Custom Accessories, Inc. v. Jeffrey-Allan Indus. Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986); *Orthopedic Equip. Co. v. United States*, 702 F.2d 1005, 1011 (Fed. Cir. 1983).

Petitioner contends that a person of ordinary skill in the art as of the relevant date would have "had an M.D. and/or a Ph.D. in immunology, biochemistry, cell biology, molecular biology, pharmaceutics, or a related discipline, with at least two years of experience in the field." Pet. 21. Patent Owner does not dispute this definition, but suggests that we interpret the relevant field as directed to "engineering monoclonal antibodies for human therapeutic use, either in the laboratory or industry." *See* Prelim. Resp. 45 (emphasis omitted).

Petitioner's proposed definition is not inconsistent with the cited prior art. Patent Owner responds, however, that it "does not dispute Amgen's POSA definition, except to clarify that the POSA would have at least two years of experience in engineering monoclonal antibodies for human therapeutic use, either in the laboratory or industry." Prelim. Resp. 45 (emphasis omitted); *see also id.* (arguing that Petitioner cannot prove unpatentability of the challenged claims under either definition).

Considering that each of the asserted references (discussed *infra*) is substantially directed to the development of monoclonal antibodies for the treatment or diagnosis of human disease, Patent Owner's proposed clarification provides useful context. *See Okajima v. Bourdeau*, 261 F.3d 1350, 1355 (Fed. Cir. 2001) ("the prior art itself [may] reflect[] an appropriate level" as evidence of the ordinary level of skill in the art)

(quoting *Litton Indus. Prods., Inc. v. Solid State Sys. Corp.*, 755 F.2d 158, 163 (Fed. Cir. 1985)). Accordingly, we define one of ordinary skill in the art as a person with an M.D. and/or a Ph.D. in immunology, biochemistry, cell biology, molecular biology, pharmaceutics, or a related discipline, with at least two years of experience in engineering monoclonal antibodies for human therapeutic use, either in the laboratory or industry. Our decision whether to institute, however, does not turn on which party's definition of the skilled artisan is used, and our determinations would be unchanged if we applied Petitioner's definition.

C. Claim Construction

Based on the filing date of the Petition (Feb. 28, 2019), the Board interprets claim terms in an *inter partes* review ("IPR") using the same claim construction standard that is used to construe claims in a civil action in federal district court. *See* 83 Fed. Reg. 51,340 (Nov. 13, 2018) (to be codified at 37 C.F.R. pt. 42).

In construing claims, district courts give claims their ordinary and customary meaning, which is "the meaning that the term would have to a person of ordinary skill in the art in question at the time of the invention." *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312–13 (Fed. Cir. 2005) (en banc). Sources for claim interpretation include "the words of the claims themselves, the remainder of the specification, the prosecution history [i.e., the intrinsic evidence], and extrinsic evidence concerning relevant scientific principles, the meaning of technical terms, and the state of the art." *Id.* at 1314 (quoting *Innova/Pure Water, Inc. v. Safari Water Filtration Sys., Inc.*, 381 F.3d 1111, 1116 (Fed. Cir. 2004)). "[T]he claims themselves [may] provide substantial

guidance as to the meaning of particular claim terms." *Id.* However, the claims "do not stand alone," but are part of "'a fully integrated written instrument,' consisting principally of a specification that concludes with the claims," and, therefore, the claims are "read in view of the specification." *Id.* at 1315 (quoting *Markman v. Westview Instruments, Inc.*, 52 F.3d 967, 978–79 (Fed. Cir. 1995)).

At this stage in the proceeding, we need only construe the claims to the extent necessary to determine whether to institute *inter partes* review. *See Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017) ("[W]e need only construe terms 'that are in controversy, and only to the extent necessary to resolve the controversy.'" (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999))).

Although at this stage of the proceeding no claim term requires express construction, for the sake of clarity, we address Petitioner's unopposed position that "immediate," as used in claim 9, encompasses events occurring within one week after administering eculizumab:

> Claim 9 recites that the claimed method "results in an *immediate* and sustained decrease in mean levels of lactate dehydrogenase (LDH)." AMG1001, claim 9. The '504 patent specification teaches "[t]he impact of terminal complement inhibition with eculizumab on chronic intravascular hemolysis in PNH patients was demonstrated in this study by an *immediate (one week)* and sustained decrease in mean levels of LDH." AMG1001, 22:22-25. Claim 10 depends from claim 9 and further requires that "the immediate decrease occurs *within one week* of administration of the antibody." AMG1001, claim 10. Thus, as Dr. Balthasar explains, a POSA would have understood the term "immediate"

> to include a decrease beginning "within one week" after administering the eculizumab. AMG1002, ¶¶64-65.

Pet. 22. Considering the above-cited portion of the Specification and the dependency of claim 10 noted in the Petition, we agree with Petitioner's assertion that "immediate" as used in claim 9 encompasses within its scope events occurring within one week after administering eculizumab.

D. Anticipation by Hillmen (Ground 1)

In Ground 1, Petitioner challenges claims 1–3 and 7–10 as anticipated by Hillmen. Pet. 25–37. Patent Owner opposes. Prelim. Resp. 45–51. For this Ground, here we focus on claim 1.

1. The Parties' Contentions

Petitioner contends "Hillmen discloses all the limitations of claim 1, either expressly or inherently, and is enabling." Pet. 25 (citing Ex. 1002 ¶¶ 75–84). In particular, Petitioner argues that Hillmen *explicitly* discloses every element of claim 1, except for eculizumab's amino acid sequence. *Id.* at 26. With respect to that amino acid sequence, Petitioner contends "Hillmen's antibody necessarily 'comprises a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4' because Alexion admitted that Hillmen's eculizumab possesses those very amino acid sequences." *Id.* at 26–27 (citing Ex. 1014, 765, 767 (Boone Declaration ¶¶ 5–6)[15]; Ex. 1024, 109; Ex. 1025, 2 (these exhibits identify a trial "CO2-

[15] Declaration Pursuant to 37 C.F.R. § 1.132 by Dr. Laural Boone, dated May 11, 2017 (submitted during the prosecution of U.S. Application No. 15/148,839, which became the '149 patent) (Ex. 1014, 763–70, "Boone Declaration").

001" as testing SOLIRIS). The Boone Declaration, cited by Petitioner, in relevant portion, states first that study C02-001 was a study of the effect of eculizumab (h5G1.1-mAb) on patients with PNH, that Dr. Boone had reviewed the eculizumab antibody used in that study and its amino acid sequence, and that Dr. Boone "concluded that the antibody (eculizumab) used in each of the studies . . . contained the heavy and light chain sequences of SEQ ID NOs: 2 and 4." Ex. 1014, 764–67 (¶¶ 5–6).

Although Petitioner does not argue that Hillmen literally and expressly disclosed the claimed antibody structure with SEQ ID NOs: 2 and 4 (which, we note, it does not), Petitioner's position is that, because Hillmen disclosed a trial of the SOLIRIS eculizumab antibody, and because Patent Owner conceded that the eculizumab antibody used is the claimed anti-C5 antibody, that Hillmen inherently discloses the claimed sequences. Pet. 27–29 (citing *In re Crish*, 393 F.3d 1253 (Fed. Cir. 2004)).

Petitioner's inherency rationale, based on "the general knowledge in the relevant field" (*id.* at 31), is summarized as follows. Contemporaneous with Hillmen's disclosure, the skilled artisan would have known that Bowdish disclosed the entire amino acid sequence of eculizumab, but for a heavy chain CDR3 region, which Bowdish disclosed as substituted with a TPO (thrombopoietin) amino acid sequence; the skilled artisan, however, would have known that Evans disclosed the amino acid sequences of eculizumab's heavy and light chain variable domains, including heavy chain CDR3 region absent from Bowdish; alternatively, the skilled artisan would have understood that Mueller disclosed the hybrid IgG2/IgG4 heavy chain and light chain constant domains of eculizumab, whereas Evans disclosed

the respective variable domains. Pet. 29–31 (citing Ex. 1004, 553–554; Ex. 1006 ¶¶ 191–193, Figs. 13A, 13B; Ex 1007, 44:4–13; Ex. 1008, 52–53, 58–61; Ex. 1002 ¶¶ 60, 81–84). With this knowledge in hand, the skilled artisan would have known that Hillman discloses a method of treatment using eculizumab—which was necessarily the claimed anti-C5 antibody with the claimed SEQ ID NOs: 2 and 4. *Id.*

Petitioner also appears to argue that Hillmen inherently discloses the hybrid IgG2/IgG4 heavy chain portion of SEQ ID. NO: 2, because one of ordinary skill in the art would have known that eculizumab contains this structure. Pet. 17 (citing Ex. 1034, 1279; Ex. 1049, 838–839; Ex. 1002 ¶¶ 46–49). Petitioner argues, for example, that Tacken explicitly describes eculizumab as containing an IgG2/IgG4 constant region. *Id.* (quoting Ex. 1034, 1279); *see, e.g.*, Ex. 1002 ¶ 46 ("Tacken confirms that eculizumab contains a hybrid IgG2/IgG4 constant region. AMG1034, 1278-1279. Tacken describes using 'h5G1.1-mAb (5G1.1, eculizamab [sic]; Alexion Pharmaceuticals)' containing an 'IgG2/IgG4 constant region.' AMG1034, 1279.").

Petitioner also relies on statements made by Patent Owner on the record during the prosecution of U.S. Patent Application Ser. No. 11/127,438, where, in arguing that disclosures upon which the applicant relied for priority were supportive of the then-pending claims, Alexion stated:

> Applicant respectfully disagrees and asserts that the priority applications provide ample written support for the claimed descriptions. For example, the priority documents each describe that "Particularly useful anti-C5 antibodies are h5G1.1, h5G1.1-

> scFv and functional fragments of h5G1.1 are *described in U.S. Patent No. 6,355,245* [Evans], the disclosures of which are incorporated herein in their entirely [*sic*] by this reference . . . Applicant submits that h5G1.1 . . . [was] *well-known to one of ordinary skill in the art as eculizumab* . . . at the time of filing of priority applications.

See Pet. 10 (quoting Ex. 1049, 838–39 (emphasis Petitioner's)). Thus, Petitioner argues, Alexion represented to the Patent Office that the antibody structure disclosed in Evans was well-known to the skilled artisan so that such a person of skill would have considered this structure to be the eculizumab in Hillmen, and which was publically disclosed before the March 15, 2007 priority date of the '504 patent.

Patent Owner takes the position that, "[p]rior to March 15, 2007, the priority date of the '504 patent, the unique amino acid sequence of SOLIRIS® was not publicly known or disclosed in the prior art." Prelim. Resp. 1.

> If a POSA were searching for the sequence of "eculizumab" as described in the art, the literature as of March 15, 2007 identified an amino acid sequence and corresponding structure that is very different from what the '504 patent claims. In particular, publications describing the safety, efficacy, and clinically relevant biological activity of "eculizumab" consistently directed a POSA to the 1996 "Thomas" publication (AMG1023) for the structure and design of the antibody, which in turn described a humanized antibody constructed with a naturally-occurring "IgG4" heavy chain constant region. The claimed antibody of the '504 patent has a very different, uniquely engineered, non-naturally occurring constant region that was nowhere described in Thomas or the prior art literature showing the safety and efficacy of "eculizumab."

Id. at 2–3 (emphasis omitted).

It is Patent Owner's position that, reading Hillmen's disclosure of "eculizumab" and Hillmen's reference to Thomas, the skilled artisan would not have been directed to the version of eculizumab encompassed by claim 1 of the '504 patent, but would have understood Hillmen to refer to Thomas's disclosed eculizumab, which is an IgG4 isotype antibody, which does not have the hybrid IgG2/IgG4 heavy chain sequence as described in SEQ ID NO: 2. *Id.* at 3, 11–13 (referencing Ex. 1023). In particular, Patent Owner notes that Hillmen cites Thomas as *the* reference for eculizumab. *Id.* at 3, 12 (citing Ex. 1004, 533 (which cites Ex. 1023 as reference "15")).

> Thomas . . . described the design and testing of a humanized anti-C5 antibody (termed "humanized 5G1.1" or "h5G1.1") featuring an "IgG4" heavy chain constant region, which was selected because the IgG4 isotype was thought to avoid activating human complement. (AMG1023 at 1396, 1399.) Thomas reported data showing that the IgG4 humanized antibody had suitable affinity and specificity, and was as effective as the original mouse antibody (termed "murine 5G1.1" or "m5G1.1") in an *in vitro* assay showing activity blocking C5 cleavage and preventing lysis of blood cells due to complement activity. (AMG1023 at 1396.)

Id. at 12–13 (emphasis omitted).

Patent Owner states that "[t]oday, but not prior to the March 15, 2007 priority date for the '504 patent, it is known that SOLIRIS® has the specific amino acid sequence recited in the claims of the '504 patent, namely, 'a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.'" *Id.* at 15 (emphasis omitted). Patent Owner argues that, as of the critical date, Hillmen did not

> enable[ ] a POSA to make and use the specific antibody recited in claims 1-3 and 7-10 without undue experimentation, because

> Hillmen guided a POSA as of March 15, 2007 to make and use a very different antibody – the ***IgG4*** isotype antibody of Thomas. *See, e.g.*, *Elan Pharm., Inc. v. Mayo Found. for Med. Educ. & Research*, 346 F.3d 1051, 1055 (Fed. Cir. 2003) (for a reference to anticipate, "[i]t is insufficient to name or describe the desired subject matter, if it cannot be produced without undue experimentation").

Id. at 46–47. Patent Owner further argues that

> [t]he mere naming of an investigational product (*e.g.*, "eculizumab") in a prior art publication does ***not*** inherently anticipate later-filed patent claims detailing the specific structure or composition of that product (*i.e.*, SEQ ID NOs: 2 and 4), if a POSA could not have ***necessarily*** determined the later claimed structure/composition from the information publicly available as of the priority date.

Id. at 48–49 (citing *Endo Pharm. Solutions, Inc. v. Custopharm Inc.*, 894 F.3d 1374, 1378–83 (Fed. Cir. 2018)).

2. Analysis

At this stage in the proceedings and for the reasons discussed below, we find Petitioner has not carried its burden to show a reasonable likelihood of anticipation of claim 1 of the '504 patent under Ground 1.

Petitioner concedes Hillmen does not expressly disclose the claimed antibody; instead, Petitioner relies on the doctrine of inherency and a post-priority-date admission by Patent Owner that the pharmaceutical (eculizumab) referenced in Hillmen was actually the claimed antibody.

Hillmen "tested the clinical efficacy of eculizumab, a humanized antibody that inhibits the activation of terminal complement components, in patients with PNH." Ex. 1004, 552. Hillmen teaches that "[e]culizumab is a recombinant humanized monoclonal antibody that was designed to block the

activation of terminal complement components.[14,15] It binds specifically to the terminal complement protein C5." *Id.* at 553. Citation "14" of Hillmen refers to Lutz Riechmann et al., *Reshaping human antibodies for therapy*, 332 NATURE 323–27 (1988). This reference is not an exhibit in this proceeding and Petitioner does not suggest that it mentions eculizumab. Citation "15" of Hillmen refers to Thomas (Ex. 1023), which discloses a monoclonal antibody (5G1.1) that recognizes the human complement protein C5, which was shown to effectively block C5 cleavage. *See* Ex. 1023, 1389.

Thomas discloses the process of developing a humanized antibody (h5G1.1 HuG4) for human C5. Thomas summarizes this work in a section titled "*Construction of a humanized h5G1.1 antibody*":

> Having demonstrated the effective humanization of the 5G1.1 variable regions, an intact humanized antibody (IgG4 isotype) was constructed and produced in 293-EBNA cells. The avidity of this humanized antibody (h5G1.1 HuG4) for human C5, was compared to the murine 5G1.1 mAb by determining the ability of each to compete binding of biotinylated 5G1.1 mAb to C5 (Fig. 9). The humanized h5G1.1 mAb had a two-fold lower avidity than the murine antibody. However, the humanized h5G1.1 HuG4 antibody was equipotent with the murine antibody at protecting PAEC from lysis by human serum, with a 0.5-fold molar ratio of antibody to C5 (1:1 ratio of antibody binding sites to C5) completely inhibiting lysis of the PAEC (Fig. 10).

Id. at 1396.[16] We find nothing in Thomas that expressly discloses or alludes

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[16] Patent Owner argues that as of the '504 patent's priority date, many other references cited Thomas when referring to eculizumab. *See* Prelim. Resp. 24–29, Table 1. Without going into detail, we find Patent Owner has accurately shown how other, contemporaneous prior art references cited Thomas for this purpose.

to a hybrid IgG2/IgG4 antibody. *See generally* Ex. 1023.

We also consider Dr. Balthasar's assertion that one of ordinary skill in the art "would have known that eculizumab contains a hybrid IgG2/IgG4 heavy chain constant region" because Tacken "describes using 'h5G1.1-mAb (5G1.1, eculizamab [sic]; Alexion Pharmaceuticals)' containing an 'IgG2/IgG4 constant region.'" Ex. 1002 ¶ 46 (citing Ex. 1034, 1279).

In seeking to address this assertion, Patent Owner takes the position that Tacken (also addressed in the context of Petitioner's obviousness challenges) is non-analogous art because it "does not involve the same field as the '504 patent, and would not have been reasonably pertinent to the particular problem addressed by the '504 patent." Prelim. Resp. 37; Sur-Reply 2–3. On the present record, we do not find Patent Owner's argument persuasive. *See In re Geisler*, 116 F.3d 1465, 1470 (Fed. Cir. 1997) (mere lawyer's arguments and conclusory statements that are unsupported by factual evidence are entitled to little probative value).

"Prior art is analogous if it is from the same field of endeavor or if it is reasonably pertinent to the particular problem the inventor is trying to solve." *Circuit Check Inc. v. QXQ Inc.*, 795 F.3d 1331, 1335 (Fed. Cir. 2015). In the present case, Tacken discloses the development of "a humanized antibody, hD1V1G2/G4 (hD1), directed against the C-type lectin DC-specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN) to explore its capacity to serve as a target receptor for vaccination purposes." Ex. 1034, Abstract. Given Patent Owner's position that the relevant field of the '504 patent encompasses the "engineering monoclonal antibodies for human therapeutic use, either in the laboratory or industry"

(*see* Section II(B), above), Tacken's development of the monoclonal antibody hD1V1G2/G4 (hD1) "for vaccination purposes" would appear to be in the same field of endeavor. Moreover, because Tacken's antibodies employ the same IgG2/IgG4 constant region as SEQ ID NO. 2 of claim 1, we disagree that Tacken is not reasonably pertinent to the '504 patent.

With reference to its hD1V1G2/G4 (hD1) antibody, Tacken discloses that, "[a]n isotype control antibody, h5G1.1-mAb (5G1.1, eculizumab; Alexion Pharmaceuticals) containing the same IgG2/IgG4 constant region, is specific for the human terminal complement protein C5." Ex. 1034, 1279. Although, as Patent Owner points out, Tacken then cites to Thomas, Patent Owner does not address why Tacken would use an eculizumab having the IgG4 constant region as set forth in Thomas as "[a]n isotype control antibody" for one having an IgG2/IgG4 constant region. Dr. Balthasar, however, testifies that Tacken also cites Mueller II[17] "as providing information about the hybrid IgG2/IgG4 constant region portion of eculizumab." Ex. 1002 ¶ 56 (citations omitted); *see* Ex. 1034, 1279 (indicating that in the construction of hD1V1G2/G4 (hD1), "humanized variable heavy and variable light regions were [ ] genetically fused with a human hybrid IgG2/IgG4 constant domain," as set forth in Mueller II (reference 17)). On the present record, we read Tacken as disclosing that humanized monoclonal antibody 5G1.1, known as eculizumab, encompasses

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[17] John P. Mueller et al., *Humanized porcine VCAM-specific monoclonal antibodies with chimeric IgG2/G4 constant regions block human leukocyte binding to porcine endothelial cells*, 34 Mol. Immunol. 441–52 (1997) (Ex. 1031).

a molecule having a hybrid IgG2/IgG4 constant domain.

That Tacken, Bowdish, and Mueller suggest that eculizumab may include an IgG2/IgG4 constant domain, however, does not necessarily lead to the conclusion that Hillmen discloses the same molecule. Rather, the present record indicates that "eculizumab" is not a single, fixed chemical entity. To the contrary, the art identified as "eculizumab" at least two such compounds that differed in the heavy chain constant region: one having the IgG4 isotype (as evidenced in Thomas), and a second having a hybrid IgG2/IgG4 isotype (as evidenced by, e.g., Tacken).

Patent Owner made the above dichotomy explicit in an official representation to the Patent Office. During Alexion's prosecution of Application No. 11/127,438, an application outside of the chain of priority of the '504 patent, but claiming the use of eculizumab for the treatment of pulmonary conditions, the Examiner took the position that "the description of 'eculizumab or pexelizumab' as well as 'the mutated Fc portions' in the priority documents is not readily apparent." Ex. 1049, 741, 830–834 (representative claim amendments). In response, Alexion asserted that

> **eculizumab was first constructed in the IgG4 isotype**, see, e.g., the bridging paragraph of the left and right columns of page 1396 of Thomas et al. (1996) [Ex. 1023], . . . **and then into the G2/G4 format**, see Mueller et al. (1997) *Molecular Immunology,* Vol. 34, No.6, pages 441-452 [Ex. 1031], . . . while **in either form** the h5G1.1 antibody was well known to be incapable to activate human complement, see, e.g., the right column, lines 27-28, of page 1399 of Thomas et al., 1996, *Id.,* and the bridging paragraphs of pages 446 to 448 and 450 to 451 of Mueller et al, 1997, *Id.* Thus, Applicant submits that it was well-known to one of ordinary skill in the art at the time of filing

> of priority applications that eculizumab has a G2/G4 Fc portion, i.e., a mutated Fe portion.

Id. at 838–839 (bolding added). Alexion's statement, above, supports both Thomas's disclosure of eculizumab in the IgG4 isotype as the first construction of eculizumab, as well as an understanding that "eculizumab" referred to and refers to a genus of antibodies, including one with the hybrid IgG2/IgG4 isotype of the challenged claims. Accordingly, in view of the record before us, we conclude that "eculizumab" referred to and refers to a class or category of anti-C5 antibodies, also called 5G1.1 or h5G1.1 mAbs.

Upon considering the facts here in view of Petitioner's reliance on *Crish*, 393 F.3d 1253, and Patent Owner's reliance on *Endo Pharms.*, 894 F.3d 1374, we find the latter case analogous here. In *Crish*, the Federal Circuit found that a claim to the nucleotide sequence of the hINV gene promoter region was inherently disclosed by prior art that "specifically identified [the promoter region] by size and location" because "[t]he starting material plasmid necessarily contain[ed] the gene of interest, including the promoter region." *Crish*, 393 F.3d at 1257–59. Here, however, Hillmen does not "specifically identify" an eculizumab antibody containing the hybrid IgG2/IgG4 sequence of SEQ ID NO: 2, as required by claim 1.

Endo Pharms. compels a different result under the facts here. In *Endo Pharms.*, the issue was again inherency (in the context of obviousness) where prior art scientific articles described clinical trials for a testosterone undecanoate composition, but did not disclose its specific, and later claimed, formulation including a particular mixture of castor oil and benzyl benzoate. *Endo Pharms.*, 894 F.3d at 1278. It was later confirmed that the

composition of the clinical trials described in the prior art articles did, indeed, have the claimed mixture of castor oil and benzyl benzoate. *Id.* The Federal Circuit held that because it was not demonstrated that a skilled artisan could extrapolate the vehicle formulation (a mixture of castor oil and benzyl benzoate) used in the prior art articles based on reported pharmacokinetic performance data, such performance could not have only been attributed to the claimed formulation and the generic disclosure of the pharmaceutical formulation in the prior art did not inherently disclose the claimed formulation. *Id.* at 1281–83. Furthermore, *Endo Pharms.* distinguished *Crish* because it was about the inherent properties of a known prior art product rather than a product that was named but not known or determinable based on the prior art disclosure of its performance characteristics. *Id.* at 1383.

In Ground 1, the prior art reference, Hillmen, discloses a clinical trial of "eculizumab" but does not otherwise identify the structure of the antibody tested, other than by referencing Thomas. Ex. 1004, 552, 553 (citing Ex. 1023). Thomas discloses a version of eculizumab different from that claimed (an IgG4 isotype rather than a hybrid IgG2/IgG4 antibody). Even accepting that a skilled artisan would have known of a hybrid IgG2/IgG4 antibody, as claimed (*see, e.g.*, Ex. 1002 ¶ 13), Hillmen's mere reference to "eculizumab" would have at least invoked a molecule having the IgG4 isotype taught in Thomas. Thus, Hillmen's disclosure of "eculizumab" would not have necessarily led the skilled artisan to the claimed antibody with "a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4." Moreover, none of the references teaching or suggesting

an IgG2/IgG4 isotype (e.g., Bowdish, Evans, Tacken, Mueller, or Mueller II) is cited in Hillmen. *See id.* at 559. We disagree with Petitioner's position that such references' teaching would have somehow overridden Hillmen's direct invocation of Thomas's disclosure of eculizumab so as to point the skilled artisan to some alternative antibody structure.[18] Accordingly, under *Endo Pharms.*, as discussed above, there is no inherent anticipation of claim 1 over Hillmen.

To summarize, as we presently understand the art as of the filing date of the '504 patent, one of ordinary skill understood that eculizumab encompassed molecules by both IgG4 isotypes and IgG2/IgG4 hybrid structures, whereas a plain reading of Hillmen suggests that it specifies the former. Accordingly, one of ordinary skill in the art would not have necessarily understood Hillmen to disclose a version of eculizumab having the IgG2/IgG4 heavy chain constant region of SEQ ID No. 2. For the above reasons, Petitioner has not demonstrated a reasonable likelihood that claim 1, the sole independent claim of the '504 patent, is unpatentable under Ground 1.

E. Obviousness in view of Hillmen, Bell, and Wang (Grounds 2 and 3)

In Grounds 2 and 3, Petitioner challenges claims 4 and 5 as obvious in view of Hillmen and Bell, and claim 6 further in view of Wang. Pet. 37–45.

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[18] We need not address here Patent Owner's non-trivial argument that Petitioner "has improperly attempted to shoehorn" the obviousness arguments of Grounds 4–7 into its anticipation contentions. Prelim. Resp. 47.

Patent Owner opposes. Prelim. Resp. 52–55. Pertinent to each of Grounds 2–7, we address Patent Owner's secondary considerations evidence in section II(H), below.

For Grounds 2 and 3, Petitioner states: "Bell disclosed a Phase 2 Pilot Study involving 11 PNH patients treated with eculizumab over a period of 12 weeks—identical to that disclosed in Hillmen. . . . Given the substantial overlap, a POSA would have had ample reason to combine Hillmen and Bell, with a reasonable expectation of success at achieving the claimed subject matter." Pet. 37–38 (citations omitted). Responding to these contentions, Patent Owner notes that, "Grounds 2 and 3 rely upon Hillmen as the sole prior art allegedly disclosing the claimed element of an antibody comprising 'a heavy chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4' . . . . [but] Hillmen did not disclose the claimed antibody sequence either expressly or inherently." Prelim. Resp. 52–53. Patent Owner's point is well taken.

As discussed in Section II(D)(2), above, Petitioner has not established that Hillmen inherently discloses the IgG2/IgG4 heavy chain constant region of SEQ ID NO: 2, and the Petition sets forth no additional argument on that subject in support of Grounds 2 and 3. Petitioner also does not argue, nor do we discern, where the required sequence is taught in Bell or Wang. Accordingly, Petitioner has not demonstrated a reasonable likelihood that claims 4–6 are unpatentable over the combinations of Hillmen, Bell, and Wang set forth in Grounds 2 and 3.

F. Obviousness in view of Bell, Bowdish, Evans, and Wang (Grounds 4 and 5)

Petitioner challenges claims 1–5 and 7–10 as obvious in view of Bell, Bowdish, and Evans (Ground 4), and claim 6 further in view of Wang (Ground 5). Pet. 45–60. Patent Owner opposes. Prelim. Resp. 56–61; *see also id.* at 55. For the purpose of institution, we focus on claim 1.

1. The Parties' Contentions

With respect to Ground 4, Petitioner contends that Bell taught all limitations of claim 1 but for eculizumab's amino acid sequence. Pet. 45–47. Petitioner points, for example, to Bell's disclosure that preferred treatments for PNH included "'an *anti-C5 antibody* selected from the group consisting of *h5G1.1-mAb (eculizumab)* . . .' suitable for treating PNH, and also that the antibody 'h5G1.1-mAb' was 'undergoing clinical trials under the tradename *eculizumab.*'" *Id.* at 46 (citing Ex. 1005 ¶¶ 12, 52). Petitioner then argues that one of ordinary skill in the art would have looked to Bowdish and Evans to obtain the amino acid sequence of eculizumab. *See* Pet. 47–54.

Petitioner contends that the skilled artisan would have looked to Bowdish for the light and most of the heavy chain sequence of eculizumab in light of Bowdish's use of an "anti-C5 antibody as the starter 'scaffold' antibody sequence for creating a recombinant TPO-mimetic" antibody. Pet. 47; Ex. 1002 ¶¶ 130–135. According to Petitioner, Bowdish disclosed "the full antibody amino acid sequence for such a 5G1.1 antibody [i.e., an anti-C5 antibody] except for the heavy chain CDR3 (HCDR3) sequence, which Bowdish replaced with the TPO-mimetic peptide sequence,

LPIEGPTLRQWLAARAPV." *Id.* (citing Ex. 1006 ¶¶ 191–193, Figs. 13A (SEQ ID NO: 67; "5G1.1 – TPO Heavy Chain (Bold Denotes TPO mimetic) Amino acid sequence"), 13B (SEQ ID NO: 69; "5G1.1 Light Chain Amino Acid Sequence"). In particular, Petitioner argues:

> A POSA . . . would have understood that the only portion of the "scaffold" 5G1.1 antibody sequence not expressly disclosed in Bowdish is the HCDR3 sequence because Bowdish taught that "[t]he TPO mimetic peptide graft in Fab clone X4b has been *transplanted into the heavy chain CDR3* of another antibody framework, *5G1.1* . . . The sequence was *cloned into 5G1.1* in such a fashion as to *replace the native CDR3*."

Pet. 50 (citing Ex. 1006 ¶ 191; Ex. 1002 ¶ 137 (emphasis Petitioner's)).

Petitioner further argues that to obtain the CDR3 sequence missing from Bowdish, one of ordinary skill in the art would have looked to Evans "because both Bell and Bowdish explicitly direct the artisan there for information on how the h5G1.1 antibody was originally created." *Id.* at 53 (citing Ex. 1002 ¶ 142; Ex. 1006 ¶ 191). Alternatively, Petitioner argues, one of ordinary skill in the art would have readily identified the entire IgG2/IgG4 heavy chain sequence from Bowdish alone, because Bowdish incorporates Evans by reference. *Id.* at 5, 18 (citing Ex. 1006 ¶ 191; Ex. 1002 ¶¶ 54–55).

According to Petitioner, "a POSA would have understood that *each* of the 5G1.1 antibody heavy chain variable regions in Evans contain *the same CDR3 sequence*: YFFGSSPNWYFDV. Thus, regardless of which 'version' of Evans' humanized 5G1.1 the POSA selected to combine with Bowdish, that heavy chain would contain the YFFGSSPNWYFDV CDR3 sequence." *Id.* at 51 (internal citations omitted) (*citing e.g.*, Ex. 1002 ¶ 136). In sum,

the "prior art would have led a POSA to make a simple substitution of one known element for another—i.e., replace the TPO-mimetic peptide sequence in Bowdish's antibody with the HCDR3 sequence from Evans—to yield predictable results: a humanized anti-C5 antibody." Pet. 53–54.

Petitioner relies on the Balthasar Declaration as support and further explanation as to how one of ordinary skill in the art would derive the claimed antibody based on the asserted art. *See* Pet. 47–51. Dr. Balthasar provides a number of illustrations useful to understanding Petitioner's argument.

Figure 4 from Dr. Balthasar's report, relating to the eculizumab light chain sequence, is reproduced below.

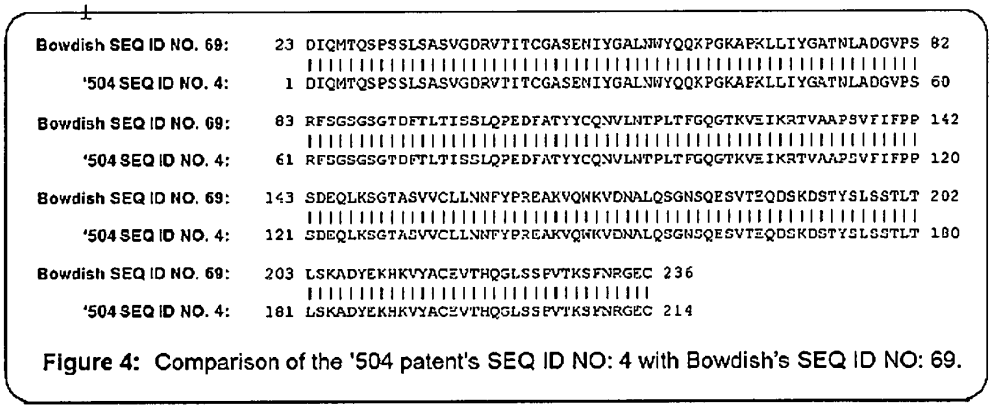

Figure 4: Comparison of the '504 patent's SEQ ID NO: 4 with Bowdish's SEQ ID NO: 69.

Ex. 1002 ¶ 52. Figure 4 purports to show identity between the mature peptide sequence[19] of the eculizumab light chain disclosed in Bowdish and

[19] Dr. Balthasar notes that Bowdish's Figures 13A and 13B identify leader sequences, not included in the comparisons of Figures 4 and 5 because one of ordinary skill in the art "would have understood that leader sequences are cleaved off as a standard part of the maturation of an antibody." *Id.*

SEQ ID NO. 4 of the '504 patent. *Id.*

Figure 5 from Dr. Balthasar's report, relating to the heavy chain sequence of eculizumab, is reproduced below.

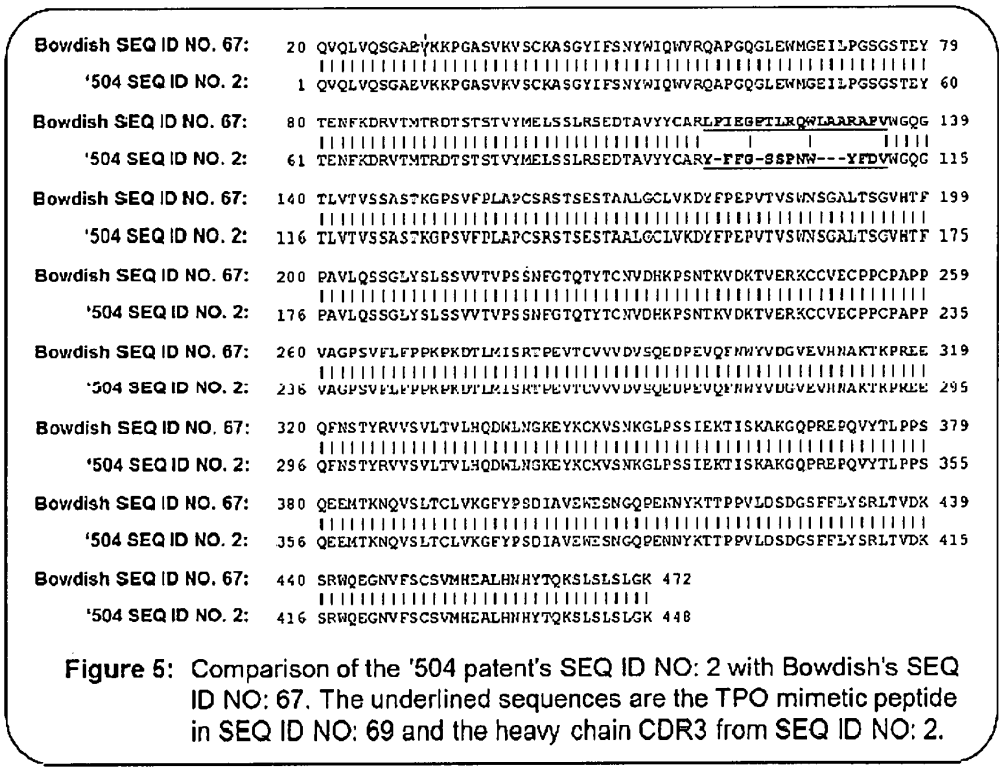

Figure 5: Comparison of the '504 patent's SEQ ID NO: 2 with Bowdish's SEQ ID NO: 67. The underlined sequences are the TPO mimetic peptide in SEQ ID NO: 69 and the heavy chain CDR3 from SEQ ID NO: 2.

Id. ¶ 55. According to Dr. Balthasar, "Figure 5 shows that the mature portion of SEQ ID NO: 67 from Bowdish (*i.e.*, amino acids 20 to 472) aligns perfectly with SEQ ID NO: 2 from the '504 patent outside of the heavy chain CDR3 region." *Id.*

Figure 6 of Dr. Balthasar's declaration, relating to the heavy chain CDRs of eculizumab, is reproduced below.

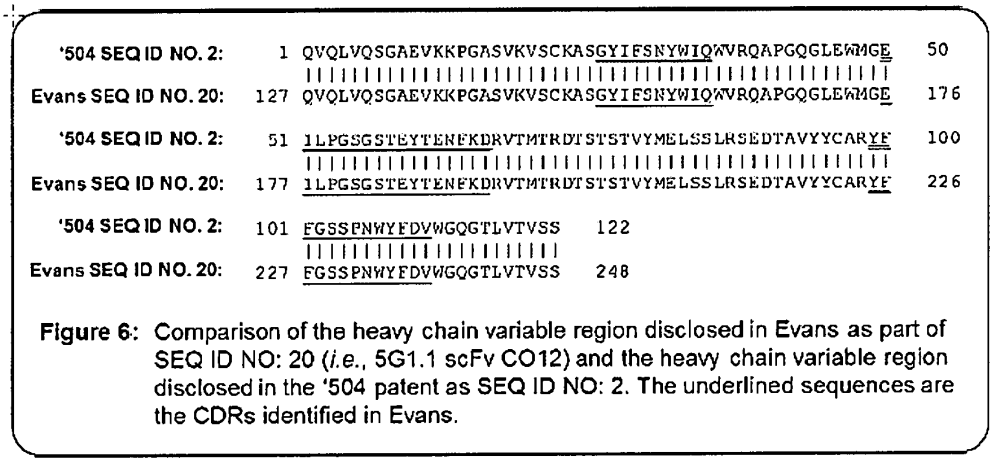

Figure 6: Comparison of the heavy chain variable region disclosed in Evans as part of SEQ ID NO: 20 (*i.e.*, 5G1.1 scFv CO12) and the heavy chain variable region disclosed in the '504 patent as SEQ ID NO: 2. The underlined sequences are the CDRs identified in Evans.

Ex. 1002 ¶ 53. According to Dr. Balthasar, Figure 6 shows an alignment of SEQ ID NO: 2 of the '504 patent with the heavy chain CDRs identified in Evans underlined, including the YFFGSSPNWYFDV sequence of CDR3. *Id.* ¶¶ 55, 136.

Figure 11 of Dr. Balthasar's declaration, relating to the relationship between the heavy chain CDR3 and Bowdish's TPO peptide, is reproduced below.

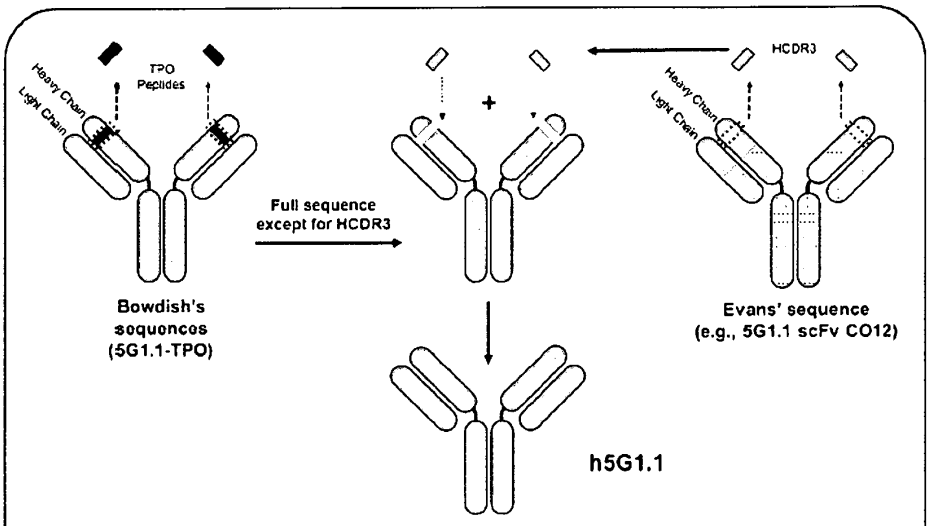

Figure 11: Diagram of the process of inserting the heavy chain CDR3 region from Evans into the 5G1.1 sequence from Bowdish to form a humanized 5G1.1 antibody.

Ex. 1002 ¶ 120. Figure 11 shows the original Bowdish scaffold antibody, including the location of eculizumab heavy chain CDR3; the use of Evans as a source of the HCDR3; and the replacement of Bowdish's TPO peptide with Evan's HCDR3. *Id.* According to Dr. Balthazar, Figure 11 "illustrate[s] how a POSA would have arrived at the claimed sequence (*i.e.*, eculizumab) by placing the heavy chain CDR3 disclosed in Evans into the 5G1.1 antibody sequence disclosed in Bowdish." *Id.*

With respect to reason to combine, Petitioner appears to argue that, because the combination of Bowdish and Bell taught the use of eculizumab for the treatment of PNH, the skilled artisan would have looked to Evans to recreate the molecule used in those successful studies. *See* Pet. 53; Ex. 1002 ¶¶ 122, 139–142. Moreover, with respect to the specific sequence of SEQ ID NO: 2, Petitioner asserts that, as of the filing date of the '504 patent, it

was well known "that antibodies with a hybrid IgG2/IgG4 constant region carried certain benefits, such as a reduced ability to elicit unwanted inflammatory events and lessened propensity to activate the complement system." Pet. 17 (citing Ex. 1032, 11, 19, 28; Ex. 1031, 451; Ex. 1002 ¶¶ 47, 57). As noted by Dr. Balthasar, for example:

> A POSA would have been aware that "the HuG2/G4 antibody design should prove useful in humanization of other antibodies intended for human use where elimination of FcR binding and C [*i.e.*, complement] activation may be desirable." AMG1031, 451; *see also*, AMG1034, 1280. Because the goal of using eculizumab in treating PNH is to reduce the level of complement activation, using a heavy chain constant region that avoids a counter-productive activation of complement by the therapeutic antibody aligns well with the desired outcome. AMG1005, ¶¶[0003], [0012].

Ex. 1002 ¶ 47.

Patent Owner argues that, absent impermissible hindsight, the skilled artisan would not have combined, or reasonably expected success in combining the asserted references. Prelim. Resp. 4–5, 56–61. Patent Owner argues that Bell, like Hillmen, taught eculizumab was the antibody of Thomas and nothing in the other references would point the skilled artisan toward a different antibody, e.g., the IgG2/IgG4 hybrid covered by the claim. *Id.* Patent Owner also argues Thomas taught away from the claimed invention because Thomas described an "eculizumab" with an IgG4 constant region. *Id.* at 57.

Patent Owner argues that even assuming one of ordinary skill did combine Bowdish and Evans to obtain eculizumab having the claimed sequence, "a POSA would not have reasonably expected the resulting

compound to work in a pharmaceutical composition for preventing C5 cleavage and safely and effectively treating PNH . . . . [because] even small changes to the amino acid sequence could have a substantial impact on the binding properties and the safety and efficacy of an antibody intended for human administration." *Id.* at 60 (citing *Novartis Pharms. Corp. v. West-Ward Pharms. Int'l Ltd.*, 923 F.3d 1051 (Fed. Cir. 2019). Patent Owner thus contends that, in view of this complexity and unpredictability, the skilled artisan would not have ventured away from Thomas's known antibody; i.e., would not have looked to Bowdish and Evans for an isotype variant of that antibody. *Id.* at 41.

2. Analysis

At this stage in the proceedings and for the reasons discussed below, we find Petitioner has carried its burden to show a reasonable likelihood that claims 1 and 6 of the '504 patent would have been obvious under Grounds 4 and 5, respectively.

Regarding Patent Owner's argument that the combination of Bell, Bowdish, and Evans involves improper hindsight and that the combination fails to render the challenged claims obvious because Bell's disclosed antibody would necessarily be that of Thomas's disclosure, we are not convinced. As summarized in Section I(F)(2), above, Bell extols the virtues of eculizumab for the treatment of PNH, but does not identify the antibody's amino acid sequence. Bell does cite to and incorporates by reference both Evans and Thomas. *See* Ex. 1005 ¶ 52. Accordingly, Thomas's IgG4 isotype antibody would be one type of eculizumab contemplated by Bell. But, as discussed in section II(D)(2), one of ordinary skill in the art would

have understood that "eculizumab" encompassed multiple isotypes, including one having the hybrid IgG2/IgG4 isotype noted in Tacken and Bowdish. Here, Bowdish discloses a substantial portion of the anti-C5 antibody 5G1.1 and points to Evans as evidencing the remaining amino acid sequence. And with respect to Patent Owner's argument that neither Bowdish nor Evans uses the term "eculizumab" (Prelim. Resp. 58–59), on the present record, we accept from Dr. Balthasar's Declaration that one of ordinary skill in the art would have looked to those references for the structure of eculizumab, particularly given that Bowdish incorporates Evans by reference for the construction of 5G1.1. *See, e.g.*, Ex. 1006 ¶ 191; Ex. 1002 ¶¶ 43–46, 51, 54, 56, 122; *see also* Ex. 1001, 39:1–32 (the '504 patent's claims also do not use the term "eculizumab"). Accordingly, Petitioner's arguments rely on the disclosures of the prior art and no improper hindsight is necessarily invoked under Petitioner's rationale.

As for Patent Owner's contention that Thomas taught away from the claimed invention (or, somehow taught away from the prior art combination), we disagree. A "teaching away" requires a reference to actually criticize, discredit, or otherwise discourage the claimed solution. *See In re Fulton*, 391 F.3d 1195, 1201 (Fed. Cir. 2004). "[T]he question is whether there is something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination, not whether there is something in the prior art as a whole to suggest that the combination is the most desirable combination available." *Id.* at 1200 (citation and emphasis omitted). "The prior art's mere disclosure of more than one alternative does not constitute a teaching away from . . .

alternatives because such disclosure does not criticize, discredit, or otherwise discourage the solution claimed." *Id.* at 1201. Thomas does not criticize, discredit, or discourage the claimed invention or the prior art combination. Thomas, at most, teaches the original eculizumab construction, an alternative to the version as taught by Bowdish and Evans.

On the present record, we are not persuaded by Patent Owner's argument that "[e]ven if Bowdish and Evans were combined . . . , a POSA would not have reasonably expected the resulting compound to work in binding to C5 or safely and effectively treating PNH" because even small changes could substantially impact an antibody's binding properties, safety, and efficacy for human administration, we disagree. *See* Prelim. Resp. 60.

As an initial matter, it is not clear whether claim 1 requires any showing of safety and efficacy as these are standards for FDA approval of new drugs rather than of patent law. *Helsinn Healthcare S.A. v. Teva Pharms. USA, Inc.*, 855 F.3d 1356, 1372–73 (Fed. Cir. 2017), *cert. granted*, 138 S. Ct. 2678 (2018), and *aff'd*, 139 S. Ct. 628 (2019) ("Approval of a new drug by FDA, however, is a more demanding standard than that involved in the patents-in-suit. The patents here make no reference to FDA standards and broadly claim a palonosetron formulation for reducing the likelihood of emesis and CINV.").

Further, Bell teaches that a variety of compounds containing the variable regions of a humanized 5G1.1 are useful to the treatment of PNH, stating: "In particularly useful embodiments, the compound is an anti-C5 antibody selected from the group consisting of h5G1.1-mAb (eculizumab), h5G1.1-scFv (pexelizumab) and other functional fragments of h5Gl.l."

Ex. 1005 ¶ 12. Moreover, Bell touts the benefits of "eculizumab" in a long-term clinical trial, indicating that the treatment is suitable for treatment of patients suffering from PNH, as set forth in claim 1. As noted above, one of ordinary skill in the art understood eculizumab to encompass a humanized 5G1.1 antibody having the IgG2/IgG4 isotype indicated in SEQ ID NO: 2.

Although we recognize Patent Owner's argument that "sequence changes outside of the antigen-binding site, *e.g.*, in the heavy chain constant region" may influence antibody affinity, specificity, and immunogenicity, this is counterbalanced by Petitioner's citation to eculizumab of the IgG2/IgG4 isotype and its reasoned argument that the skilled artisan would look to the hybrid sequence because it was known "that antibodies with a hybrid IgG2/IgG4 constant region carried certain benefits, such as a reduced ability to elicit unwanted inflammatory events and lessened propensity to activate the complement system." *See* Prelim. Resp. 11; Pet. 17 (citing Ex. 1032, 11, 19, 28; Ex. 1031, 451; Ex. 1002 ¶¶ 47, 57).

On balance, the present record suggests that a person of ordinary skill in the art would have reasonably expected a version of eculizumab having a heavy chain consisting of SEQ ID NO: 2 to work for the purpose set forth in claim 1 ("treating a patient suffering from paroxysmal nocturnal hemoglobinuria (PNH)"). The parties are invited to further address this issue during trial.

We take note of Patent Owner's arguments relating to and evidence of objective indicia of non-obviousness, discussed above at Section II(E). Although we noted that there was some evidence to support Petitioner's contentions of commercial success, long-felt but unmet need, and industry

praise, we also noted some potential shortcomings in Patent Owner's presentation, including the rebutability of the presumption of a nexus between their evidence and the claimed and novel subject matter. We expect this issue will be further developed at trial.[20]

Again, to summarize, based on the evidence presented at this stage in the proceedings, it has been shown that there is a reasonable likelihood that claim 1 of the '504 patent would have been obvious over Bell, Bowdish, and Evans under Ground 4. With respect to Ground 5, which challenges only dependent claim 6, we await further development of the parties' positions in the Patent Owner Response and Petitioner's Reply.

G. Obviousness in view of Bell, Evans, Mueller, and Wang (Grounds 6 and 7)

Petitioner challenges claims 1–5 and 7–10 as obvious in view of Bell, Evans, and Mueller (Ground 6), and claim 6 further in view of Wang (Ground 7). Pet. 61–73. Patent Owner opposes. Prelim. Resp. 61–63; *see also id.* at 55. For the purpose of institution, we focus on claim 1.

1. The Parties' Contentions

In sum, Petitioner asserts, "the only element of claim 1 not expressly taught in Bell is that its humanized anti-C5 antibody 'comprises a heavy

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[20] In addition to challenging Petitioner's positions with respect to claim 1, Patent Owner also raises non-trivial arguments in response to Petitioner's contentions specific to dependent claims 5 and 6, which we need not address at this stage of the proceeding. *See* Pet. 39–45 (citing e.g., Ex. 1005 ¶ 58, 89–96; Ex. 1002 ¶¶ 104–109), 60–61 (citing Ex. 1027, 1638 (¶ 3.5), Ex. 1004 (¶ 5.3.9); Ex. 1028, Fig. 10, ¶ 67; Ex. 1029, Table 1; Ex. 1030, Table 1; Ex. 1002 ¶¶ 168; Prelim. Resp. 55, 60–61.

chain consisting of SEQ ID NO: 2 and a light chain consisting of SEQ ID NO: 4.'" Pet. 62. According to Petitioner, "Evans disclosed the complete amino acid sequences of the heavy and light chain variable domains of a humanized anti-C5 antibody," whereas "Mueller disclosed the amino acid sequence of a light chain constant region and the hybrid IgG2/IgG4 heavy chain constant region." *Id.* at 62–63 (citing Ex. 1007, 44:4–13, SEQ ID NO: 20; Ex. 1002, ¶¶172–173, 183–185; Ex. 1008, 58–61). Petitioner further argues:

> Knowing that chimeric IgG2/IgG4 constant regions that were known not to activate the complement system (AMG1008, 7:28-31, 8:23-26, 12:27-32), a POSA reading Bell and Evans also would have looked to Mueller for "h5G1.1" sequence information. Mueller taught methods for making "chimeric antibodies containing the C1 and hinge region of human IgG2 and the C2 and C3 regions of human IgG4 . . . (HuG2/G4 mAb)." AMG1008, 12:27-30; *see also, id.,* 8:23-26. In particular, Mueller described a control antibody "h5G1.1 CO12 HuG2/G4 mAb," which a POSA would have readily identified as a humanized anti-C5 antibody because of the "h5G1.1" nomenclature coupled with the hybrid IgG2/IgG4 constant region ("HuG2/G4"). AMG1008, 12:37, FIG. 15; AMG1005, ¶[0052]; AMG1034, 1279; AMG1049, 838-839; AMG1002, ¶190.

Id. at 64.

Figure 14 from the Balthasar Declaration is reproduced below.

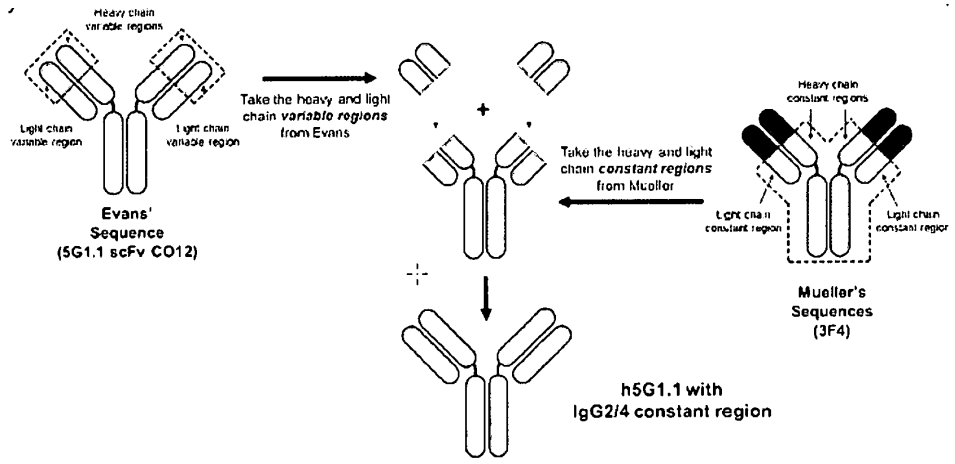

Figure 14.

Ex. 1002 ¶ 173. According to Petitioner, the above figure presents an overview of how one of ordinary skill in the art would have combined the teachings of Evans and Mueller to arrive at the eculizumab antibody described in claim 1. *Id.*

Relying on Dr. Balthasar's testimony, Petitioner argues that "Mueller disclosed the amino acid sequence of a hybrid IgG2/IgG4 heavy chain constant domain when Mueller disclosed the sequence of the chimeric anti-VCAM '3F4' antibody." Pet. 64 (citing Ex. 1002 ¶ 186; Ex. 1008, 58–61). According to Petitioner, one of ordinary skill in the art would have aligned Mueller's chimeric 3F4 HuG2/G4 heavy chain and mature 3F4 heavy and light chain variable regions such that a skilled artisan aligning the two would identify the 3F4 variable regions (at Figure 9) as amino acids 20–137 of the 3F4 HuG2/G4 heavy chain and amino acids 20–131 of the 3F4 light chain. *Id.* at 65 (citing Ex. 1008, 51–53, 58–61, Figure 9; Ex. 1002 ¶ 186).

Petitioner contends that the skilled artisan "would have immediately known that the remainder of the 3F4 HuG2/G4 heavy chain (amino acids 138-463 is the hybrid IgG2/IgG4 constant region of that antibody, and that the remainder of the 3F4 chain (amino acids 132–238) is the light chain constant region of that antibody." *Id.* at 65–66 (citing Ex. 1002 ¶¶ 179, 186, Figure 15; Ex. 1008, 52–53, 56–57). With this understanding of the heavy and light chain constant domain sequences in mind, Petitioner contends the skilled artisan would have looked to Evans to complete the whole antibody using Evans's variable regions identified from its SEQ ID NO: 20, particularly because Evans uses the same "CO12" nomenclature to refer to its 5G1.1 scFv as Mueller does in referring to h5G1.1. *Id.* at 66–67 (citing Ex. 1002 ¶¶ 173, 180–182).

Petitioner's argued rationale for combining Mueller and Evans is that Mueller taught antibodies with lower immune response and identified an antibody as h5G1.1 CO12 HuG2/G4 mAb, which the skilled artisan would have known is eculizumab. Further, Petitioner argued, Evans taught the complementary parts of this anti-C5 antibody, so, by combining the elements of the two references, a complete antibody would have been created having the SEQ ID NO: 2 and SEQ ID NO: 4 of the claim. *Id.* at 64; *see also id.* at 69–70 (further discussing why one of ordinary skill in the art would have combined the cited references with a reasonable expectation of success).

Patent Owner argues "[a] POSA would have understood that Mueller could have used ***any*** antibody with an IgG4 or IgG2/G4 isotype as a 'negative control' for its *in vitro* experiments, as long as it did not bind to

VCAM," meaning, there would be no reason to incorporate the variable regions taught by Evans. *Id.* at 62–63. And, without improper hindsight, Mueller and Evans would not have been combined by the skilled artisan. Prelim. Resp. 61. Patent Owner argues "[a] POSA as of March 15, 2007 considering the problem addressed by the '504 patent – developing an antibody that prevents cleavage of C5 and can safely and effectively treat patients suffering from PNH– would have had no reason to look at Mueller, which had nothing to do with that problem." *Id.* (citing *Broadcom Corp. v. Emulex Corp.*, 732 F.3d 1325, 1334 (Fed. Cir. 2013) ("While a prior art reference may support any finding apparent to a person of ordinary skill in the art, prior art references that address different problems may not, depending on the art and circumstances, support an inference that the skilled artisan would consult both of them simultaneously.").

2. Analysis

In contrast to Grounds 4 and 5, where Bowdish expressly incorporated Evans by reference, thereby rendering the combination unquestionable, in Grounds 6 and 7 we find no express link between the teachings of Mueller and Evans. Although such a combination is possible, the mere fact that prior art can be combined does not establish that one of ordinary skill would have done so. *See, e.g.*, *In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1992) (The "mere fact that the prior art may be modified in the manner suggested . . . does not make the modification obvious unless the prior art suggested the desirability of the modification.").

Grounds 6 and 7 present a close question on whether there would have been motivation to combine Mueller and Evans in the manner argued by Petitioner. Upon review of the Balthasar Declaration, it is apparent that Mueller's 3F4 heavy chain provides a match for part of the claimed SEQ ID NO: 2. *See* Ex. 1002 ¶ 58, Figure 8. Further, Mueller's 3F4 light chain provides a match for part of the claims SEQ ID NO: 4. *Id.* ¶ 59, Figure 9. The Balthasar Declaration provides an illustration as its Figure 10, reproduced below, showing the extent that each of Evans and Mueller (and Bowdish, per Grounds 4 and 5) discloses the claimed SEQ ID NOs: 2 and 4.

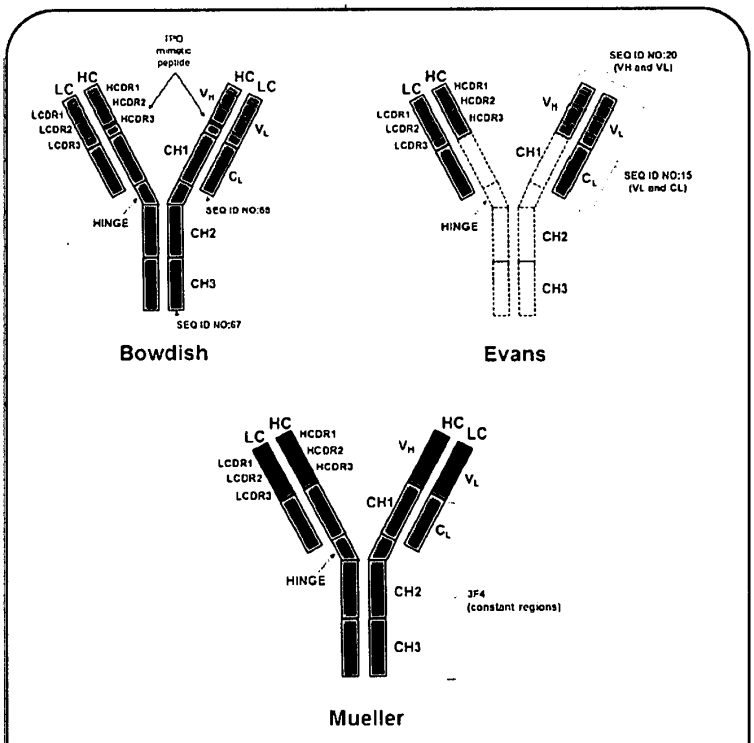

Figure 10: Diagram of the portions of the claimed antibody sequence disclosed in Bowdish (SEQ ID NOs: 67 and 69), Evans (SEQ ID NOs: 15 and 20), and Mueller (as part of the 3F4 antibody). *Green*: sequences matching SEQ ID NOs: 2 and 4 from the '504 patent; *blue*: other sequences

Ex. 1002 ¶ 60. Dr. Balthasar's Figure 10 shows three antibody structures: Bowdish top-left, Evans top-right, and Mueller bottom center. The Figure shows identity with SEQ ID NOs: 2 and 4 of the '504 patent in green and differences in blue.

Based on the above figure, Petitioner's rationale for combining the teachings of Mueller and Evans is somewhat tenuous. In particular, we are concerned with the proposed reasons one of ordinary skill in the art would have paired Evans with Mueller, choosing precisely those portions of Evans's and Mueller's constructs to create an antibody having exactly the sequences set forth SEQ ID NOs: 2 and 4. At this stage in the proceedings, based on the evidence before us, the answer is not entirely clear and Patent Owner's argument regarding improper hindsight makes some sense. True, Mueller discloses "a humanized antibody directed against human C5 (h5G1.1 CO12 HuG4 mAb)," but little else regarding its structure. *See* Ex. 1008, 12. It is not apparent that the skilled artisan, knowing of Evans, would have looked to Mueller, or vice versa.

Based on our understanding of the evidence presented at this stage in the proceedings, Petitioner has not sufficiently demonstrated a reasonable likelihood that claim 1 of the '504 patent would have been obvious over the combinations set forth in Grounds 6 and 7.

H. Objective Evidence of Non-Obviousness

Factual considerations that underlie the obviousness inquiry include the scope and content of the prior art, the differences between the prior art and the claimed invention, the level of ordinary skill in the art, and any

relevant secondary considerations. *See Graham*, 383 U.S. at 17–18. Relevant secondary considerations include commercial success, long-felt but unsolved needs, failure of others, and unexpected results. *KSR*, 550 U.S. at 406, (2007). Although evidence pertaining to secondary considerations must be taken into account whenever present, it does not necessarily control the obviousness conclusion. *See, e.g., Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1372 (Fed. Cir. 2007).

Petitioner contends no objective indicia of nonobviousness support patentability of the challenged claims. Pet. 37, 38, 45, 60–62, 71–73. In particular, Petitioner notes that during the prosecution of the '504 patent, Alexion argued that the claimed heavy chain of eculizumab, i.e., the hybrid IgG2/IgG4 constant domain, provided surprising and unpredictable results, such as decreased effector function, reduced immunogenicity, and increased half-life. Pet. 72 (citing Ex. 1014, 588, 593 (¶ 8)). However, Petitioner contends, because eculizumab's hybrid IgG2/IgG4 constant region was well known in the art (as evidenced by Tacken), the allegedly surprising and unpredictable features of the antibody have no nexus to the challenged claims. *Id.* (citing Ex. 1034, 1279).

Petitioner further contends that one of ordinary skill in the art would not have found surprising the alleged beneficial results of using the hybrid constant region because, in view of Mueller II, antibodies with the claimed hybrid IgG2/IgG4 heavy chain were known not to bind FcR and to be less immunogenic, whereas it was well known that antibodies with this claimed hybrid heavy chain would have an increased half-life. *Id.* (citing Ex. 1031,

488, 451; Ex. 1032 ("Rother"), 5, 19[21]; Ex. 1002 ¶ 210–212); *see, e.g.*, Ex. 1032, 5–6 (disclosing that antibodies having "an engineered constant region that includes an IgG2-derived portion and an IgG4-derived portion . . . maintain the function of the non-Fc component and/or have increased half-life compared to the non-Fc component alone and/or lack unwanted antibody Fc-mediated cell activating and inflammatory properties including events resulting from Fc-receptor antibody engagement and complement activation").

Patent Owner contends that evidence of commercial success, long-felt, but unmet need, and industry praise support the non-obviousness of the challenged claim. Prelim. Resp. 63. Patent Owner argues that SOLIRIS, the product embodying the claimed antibody, is a commercial success, having annual net product sales in excess of $1 billion in 2018. *Id.* at 64. (citing Ex. 2018, 70). Patent Owner further contends that this commercial success "has a direct nexus to the patented features of the '504 patent, which claims the uniquely-engineered, non-naturally occurring antibody responsible for the drug's clinical (and therefore commercial) success as a treatment for PNH, as well as complement-mediated hemolytic condition aHUS." *Id.*

At this stage in the proceedings, based on the evidence presented by Patent Owner, it is apparent that SOLIRIS is a successful product. "[T]here is a presumption of nexus for objective considerations when the patentee shows that the asserted objective evidence is tied to a specific product and that product "is the invention disclosed and claimed in the patent." *WBIP,*

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[21] Rother et al., WO 2005/007809 A2, published Jan. 27, 2005 (Ex. 1032).

LLC v. Kohler Co., 829 F.3d 1317, 1329 (Fed. Cir. 2016). This "presumption of nexus is rebuttable: a patent challenger may respond by presenting evidence that shows the proffered objective evidence was 'due to extraneous factors other than the patented invention.'" *Id.* Here, the parties appear to agree that the claim of the '149 patent is directed to the commercial product SOLIRIS. However, commercial success "is relevant in the obviousness context only if there is proof that the sales were a direct result of the unique characteristics of the claimed invention – as opposed to other economic and commercial factors unrelated to the quality of the patented subject matter." *In re Huang*, 100 F.3d 135, 140 (Fed. Cir. 1996).

Patent Owner argues that because SOLIRIS is "the first FDA-approved treatment to reduce hemolysis in patents with PNH," there is evidence that the claimed antibody fulfilled a long-felt, unmet need in the market. Prelim. Resp. 64 (citing Ex. 2019, 1270).

At this stage in the proceeding, the available evidence supports that anti-C5 antibodies were considered potential therapeutic options for "many years" before 2007, and that Alexion's eculizumab product "is currently the only complement-specific antibody on the market" and is the "first and only approved therapy for PNH." Ex. 2019, 1270. Again, it may be presumed that there is a nexus between the claimed and novel elements of the SOLIRIS product and the meeting of the long-felt need. However, "[w]here the offered secondary consideration actually results from something other than what is both claimed and *novel* in the claim, there is no nexus to the merits of the claimed invention." *In re Kao*, 639 F.3d 1057, 1068 (Fed. Cir. 2011).

Patent Owner also contends "SOLIRIS® also received industry praise as the recipient of multiple Prix Galien awards (the industry's highest accolade . . . ." Prelim. Resp. 64--65 (citing Ex. 2020; Ex. 2021).

As with the other two contended bases for indicia of non-obviousness, while it is apparent there was high praise for the SOLIRIS product from the relevant industry, there is a rebuttable presumption that this praise has a nexus with the claimed subject matter. *Cf. In re Kao*, 639 F.3d at 1068.

Given the early stage of these proceedings, we decline to accord much weight to Patent Owner's substantially untested evidence of objective indicia of nonobviousness. The parties will have the opportunity to further develop these facts during trial, and the Board will evaluate the fully-developed record at the close of the evidence.

I. The Board's Discretion to Deny Institution under 35 U.S.C. §§ 325(d) and 314(a)

1. The Parties' Positions

Patent Owner argues the "Petition should also be denied institution under 35 U.S.C. §§ 325(d) and 314(a), because Amgen's Grounds rely on the 'same or substantially the same prior art or arguments' previously presented to the PTO" "[i]n the course of patent prosecution leading to issuance of the '504 patent, as well as prosecution of related U.S. Patent Nos. 9,719,880 ('the '880 patent') and 9,732,149 ('the '149 patent'). Prelim. Resp. 17, 65–66; *see id.* at 65–66 (asserting that the Examiner considered Hillmen, Evans, and Wang; and further reviewed Bell ("cumulative of Bowdish"), and Mueller II ("cumulative of Mueller").

Patent Owner argues that in the course of prosecution leading to the issuance of the '504 patent, the Examiner:

- Expressly discussed Amgen's asserted references Hillmen 2004 (AMG1004), Evans (AMG1007), and Wang (AMG1028) as a basis for rejection, before ultimately finding the claims to be allowable over the art (*see, e.g.*, AMG1014 at 557-561, 623-628, 738-743);
- Considered Amgen's asserted reference Bell (AMG1005), which Alexion submitted to the PTO (*see, e.g.*, AMG1014 at 566);
- Considered U.S. Patent No. 7,482,435 (ALXN2016), which is the parent to and cumulative of Amgen's cited "Bowdish" application (AMG1006), disclosing the same information on which Amgen relies here (*see, e.g.*, AMG1014 at 565); and
- Considered the "Mueller II" article (AMG1031), which is cumulative of Amgen's asserted "Mueller" reference (AMG1008) because, as Amgen's declarant recognized, it "discloses the same antibodies" as Mueller. (*See, e.g.*, AMG1014 at 499; AMG1002 ¶ 182 & n.14.)

Prelim. Resp. 18.

Petitioner's position on this issue is that "[t]he arguments and evidence presented herein were not before the examiner during prosecution and, therefore, do not constitute 'the same or substantially the same prior art or arguments' under 35 U.S.C. §325(d).'" Pet. 24. In particular, Petitioner argues that

> the examiner rejected Alexion's claims as (i) anticipated by Hillmen in view of Thomas; (ii) obvious over Hillmen, Thomas and Evans; and (iii) obvious over Hillmen, Thomas, Evans, and Wang. AMG1014, 557-561. Those rejections rested solely on disclosures in Thomas and Evans for eculizumab sequence information. *Id.* The examiner later allowed the '504 patent

> claims mistakenly believing—because of Alexion's mischaracterization of the art—that the sequence and structure of eculizumab were not already known.
>
> Though Hillmen, Evans, and Wang were referenced by the examiner during prosecution, this Petition presents them in a different light, along with new references—Bell, Bowdish, and Mueller, which teach the IgG2/IgG4 constant region missing from the art combination raised during prosecution.
>
> Bell and a parent application to Bowdish (US 2003/0049683 A1) was cited but not relied upon during prosecution, and Mueller was not cited at all. The art combinations here, which were not raised by the examiner during prosecution, provide the complete sequence of eculizumab, thereby teaching the very thing the examiner mistakenly concluded was missing from the prior art. Consequently, this Petition is not the same/substantially the same as or cumulative of any previous arguments and § 325(d) does not preclude instituting this Petition.

Id. at 24–25.

2. Analysis

Regarding the Board's discretion under 35 U.S.C. § 325(d), in *Becton, Dickinson & Co. v. B. Braun Melsungen AG*, the Board enumerated non-exhaustive factors to be considered in exercising discretion under 35 U.S.C. § 325(d) on whether to institute *inter partes* review. Case IPR2017-01586, slip op. at 17–18 (PTAB Dec. 15, 2017) (Paper 8) (precedential as to § III.C.5, first paragraph). The non-exhaustive *Becton* factors are:

> 1. the similarities and material differences between the asserted art and the prior art involved during examination;
>
> 2. the cumulative nature of the asserted art and the prior art evaluated during examination;

> 3. the extent to which the asserted art was evaluated during examination, including whether the prior art was the basis for rejection;
>
> 4. the extent of the overlap between the arguments made during examination and the manner in which Petitioner relies on the prior art or Patent Owner distinguishes the prior art;
>
> 5. whether Petitioner has pointed out sufficiently how the Examiner erred in its evaluation of the asserted prior art; and
>
> 6. the extent to which additional evidence and facts presented in the Petition warrant reconsideration of the prior art or arguments.

Id. (numbering added). The *Becton* factors are not dispositive, but are part of a balanced assessment of the relevant circumstances in a particular case and we do not simply default to a tally of each factor to determine whether or not an IPR should be instituted.

Here, Patent Owner has not clearly identified how its arguments fall under the above-noted factors, but has generally argued that the prior art before us now was considered by the prosecuting Examiner either directly or as being cumulative of references that were so considered, and has further argued that the unpatentability issues presented in the Petition are the same as those at issue before the Examiner. Upon review of this evidence, we note that the Examiner considered Hillmen in rejecting a claim for obviousness-type double patenting and for anticipation. *See* Ex. 1014, 482, 557–561. As discussed above, at this stage in the proceedings, the anticipation Ground 1 over Hillmen, on its own, is not considered sufficient to institute IPR; therefore, Hillmen's consideration during prosecution is not determinative here. Rather, we focus on the Bell, Bowdish, and Evans—the references of Ground 4, upon which we base our institution decision.

As an initial matter, Alexion did address the substance of Evans during prosecution. *See* Ex. 1014, 588. But, according to Patent Owner, the Examiner "[c]onsidered" Bell and "U.S. Patent No. 7,482,435 (ALXN2016), which is the parent to and cumulative of Amgen's cited "Bowdish" application (AMG1006), disclosing the same information on which Amgen relies here." Prelim. Resp. 18. Both of these citations, however, are to an Information Disclosure Statement signed by the Examiner. *See id.* (citing Ex. 1014, 565, 566). Patent Owner does not direct us to, nor do we discern, where either Bell or some version of Bowdish was substantively considered during prosecution. The Board has consistently declined exercising its discretion under Section 325(d) when the only fact a Patent Owner can point to is that a reference was disclosed to the Examiner during the prosecution. *See, e.g.*, *Amneal Pharms. LLC v. Alkermes Pharma Ireland Ltd.*, IPR2018-00943, slip op. at 40 (PTAB Nov. 7, 2018) (Paper 8) (declining to deny institution based on Section 325(d) where the reference was listed on the face of the patent, but Patent Owner provided no evidence "about the extent to which the Examiner evaluated" the reference during prosecution); *Digital Check Corp. d/b/a ST Imaging v. E-Imagedata Corp.*, IPR2017-00178, slip op. at 12–13 (PTAB Apr. 25, 2017) (Paper 6) (acknowledging that a prior art reference was cited in an IDS, but granting institution because there was no indication that the claims were rejected based on those references or that the Examiner substantively discussed those references during prosecution); *Fox Factory, Inc. v. SRAM, LLC*, IPR2016-01876, slip op. at 7–9 (PTAB Apr. 3, 2017) (Paper 8) (refusing to deny institution based on Section 325(d) for grounds based on a prior art reference that was simply cited in an IDS and

not considered at any length); *Praxair Distribution, Inc. v. INO Therapeutics LLC*, IPR2015-00893, slip op. at 8 (PTAB Sept. 22, 2015) (Paper 14) (granting institution even though the references were previously cited in an IDS because patent owner failed to identify with specificity where the references were considered); *HyperBranch Medical Technology, Inc. v. Confluent Surgical, Inc.*, IPR2018-01099, slip op. at 17 (PTAB Nov. 27, 2018) (Paper 14) (instituting IPR because, *inter alia*, "[t]he Examiner does not appear to have considered the combined teachings of Spero and Haber during examination of the '021 patent.").

Based on the evidence presented by Patent Owner, *Becton* factors 1–6 weigh in favor of not exercising our discretion not to institute here. Therefore, based on the evidence cited by Patent Owner and for the reasons above, we decline to exercise our discretion under section 325(d) to deny institution here.

Other than the heading of Section VI of the Preliminary Response and that section's first sentence invoking the statute, Patent Owner presents no arguments or evidence directed to the Board's discretion under 35 U.S.C. § 314(a). *See* Prelim. Resp. 65–66. *Therefore*, we also decline to exercise our discretion under Section 314(a) to deny institution.

III. CONCLUSION

On the record before us at this stage in the proceeding, Petitioner has demonstrated a reasonable likelihood of prevailing on Ground 4 in showing that claim 1 of the '504 patent is obvious over the combination of Bell, Bowdish, and Evans. Given this determination, we institute trial on all

challenged claims raised in the Petition.[22] *See PGS Geophysical AS v. Iancu*, 891 F.3d 1354, 1360 (Fed. Cir. 2018) (indicating that a decision whether to institute an *inter partes* review "require[s] a simple yes-or-no institution choice respecting a petition, embracing all challenges included in the petition").

Our decision at this stage derives from our preliminary review of the challenged claims, the asserted prior art, and the opinions set forth in the as-yet-unrebutted Balthasar Declaration. We emphasize that at this stage of the proceeding, we have not made a final determination as to the construction of any claim term or the patentability of the instituted claims. Our final decision will be based on the full record developed during trial.

IV. ORDER

Accordingly, it is hereby:

ORDERED that, pursuant to 35 U.S.C. § 314, an *inter partes* review of claims 1–10 of U.S. Patent No. 9,725,504 B2, in accordance with each ground on which the challenge to each claim is based in the Petition, is hereby *instituted*; and

FURTHER ORDERED that, pursuant to 35 U.S.C. § 314(c) and 37 C.F.R. § 42.4(b), *inter partes* review of the '504 patent will commence on the entry date of this Order, and notice is hereby given of the institution of a trial.

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[22] In view of the complexity of the art and arguments presented, the parties are, nevertheless, invited to negotiate an agreement to focus on some subset of the asserted claims and Grounds.

For PETITIONER:

Deborah A. Sterling
David H. Holman
Scott A. Schaller
David W. Roadcap
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
dsterling-PTAB@ sternekessler.com
dholman-PTAB@sternekessler.com
sschalle-PTAB@ sternekessler.com
droadcap-PTAB@sternekessler.com

For PATENT OWNER:

Gerald J. Flattmann, Jr.
Lori A. Gordon
Vanessa Y. Yen
Evan D. Diamond
James T. Evans
KING & SPALDING LLP
gflattmann@kslaw.com
lgordon@kslaw.com
jevans@kslaw.com